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CILIA AND FLAGELLA

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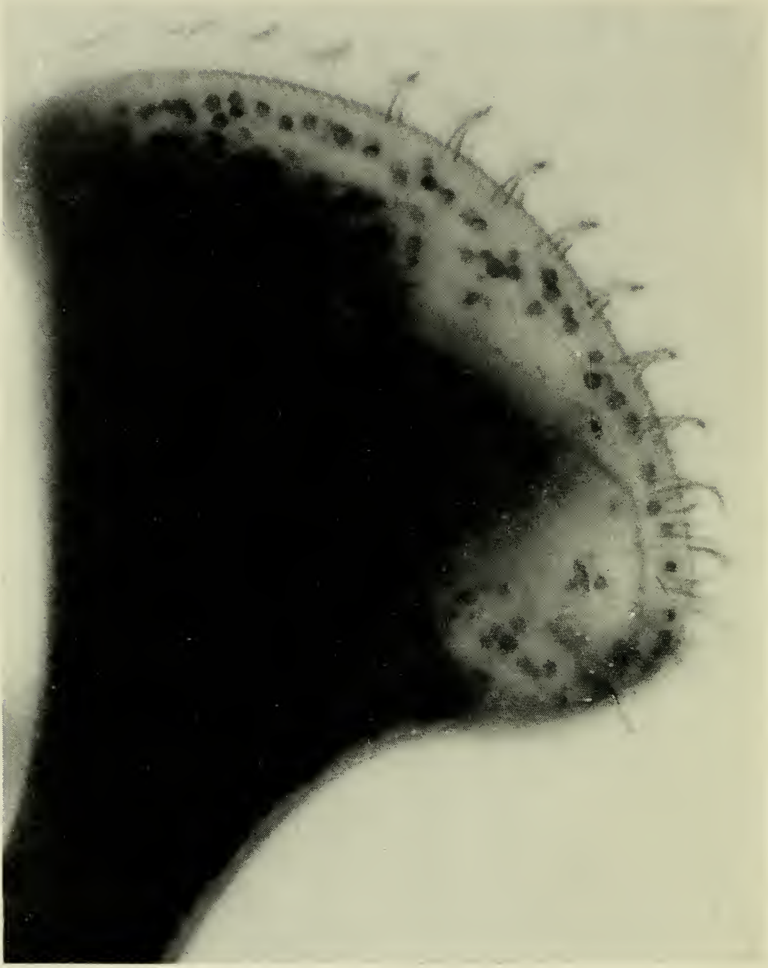

Frontispiece

Metachronal waves of membranelles of *Stentor polymorphus*

THE BIOLOGY OF
CILIA AND FLAGELLA

BY

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. . . the general existence of the ciliary motion in the Animal Kingdom is already sufficiently established, . . . whoever has opportunities and inclination to cultivate this field of inquiry will find his labour rewarded by much curious and interesting discovery.

Dr. W. Sharpey, 1835

PREFACE

IMPORTANT advances in our knowledge of the structure and physiology of cilia have been made since Gray's classical monograph on *Ciliary Movement* was published in 1928. In particular, the improvement of techniques of electron microscopy in the last decade gave rise to a renewed interest in the structure of cilia which has been rewarded with the discovery of a widespread and consistent fibril pattern in all true cilia, flagella and sperm tails. A parallel growth of interest in the physiology of the beating and co-ordination of cilia has been taking place in these years, and has been given an added stimulus by the success of morphological studies. The primary purpose of this book is to review advances in these fields since 1928, for in these 34 years no attempt has been made to summarize fundamental work on cilia and flagella in a comprehensive way. A review by J. A. Rivera entitled *Cilia, Ciliated Epithelium and Ciliary Activity* was published while the present book was in the press; Rivera approached the subject in an entirely different way in summarizing the effects of various agents on ciliary activity from the point of view of the human physiologist, so that the two reviews scarcely overlap at all. In the present book modern ideas on structure are linked with modern ideas on physiology in an attempt to make a single coherent story.

The field of study has been divided into four parts: ciliary structure, the effects of various agents on ciliary activity, the physiology of beating of cilia and the physiology of co-ordination. The second of these is intended for reference and for the introduction of evidence used in the third and fourth parts. Summaries of the material included will be found at the beginning of Chapter II (structure of a typical cilium) and at the ends of Chapters IV (beating activity) and V (co-ordination).

The literature contains many thousands of references to ciliary structure and functioning, and, in order not to overload the book with bibliography, some selection had to be made. Only some 400 references are mentioned here, recent work being cited more fully (well over half the references date within the last 10 years), while key works are quoted from older studies to enable interested students to follow up other references.

While the review was written to satisfy the needs of the honours student of Zoology, students in other fields should also find it valuable. Research workers studying any topic related to ciliary organelles or their activity should find it useful as a fairly concise summary of the structure and physiology of cilia, particularly since most reviews tend to concentrate on either structure or physiology with little reference to the other part of the subject. Where good evidence is available I have tried to keep closely to established facts, but where our knowledge is very scanty I have tended to stray into the realms of speculation; I hope that the reader will be able to distinguish facts from speculative suggestions.

Few can fail to be convinced of the need for such a review at this time, and I hope that this attempt at a summary of the present position will satisfy the requirements of as many people as possible. If any of the ideas presented here will lead others to further research, then the author will be well satisfied that he has not laboured in vain.

ACKNOWLEDGEMENTS

I am pleased to record my thanks to Professor J. E. Harris and Dr. J. A. Kitching of the University of Bristol, both for introducing me to the study of ciliary activity and for valuable comments on parts of the text of this book. I am also grateful for the advice and criticism of Professor J. O. Corliss of the University of Illinois, who, while visiting Professor of Zoology at the University of Exeter, has read the whole text and spent much time in fruitful discussions. My wife Peggy has given invaluable help in correcting manuscripts and proofs and in innumerable other ways. The help of these kind people has improved this book considerably, but the author must bear the responsibility for the shortcomings that remain.

The book was written at the suggestion of Dr. G. A. Kerkut, to whom I am sincerely indebted. My thanks are also due to Dr R. Barton, Professor J. T. Randall and Dr. K. Vickerman who have passed on valuable information for this book in discussions of their unpublished work.

It is a pleasure to acknowledge the assistance of Mr. N. Barratt in preparing photographs of some of the illustrations, and of Miss B. Mortimer in typing parts of the script.

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CHAPTER 1

INTRODUCTION

Cilia seem to have been seen for the first time by the Dutch microscopist Leeuwenhoek in 1675. In a letter to the Royal Society (Leeuwenhoek, 1677), he described the incredibly thin feet or little legs by means of which a small animalcule, which Dobell (1932) believes to have been a ciliated protozoon, moved through the water. Cilia, or the immediate result of their movements, were seen in metazoa at about the same time by de Heide (1684), who described a " motus tremulus " of the gill surface of *Mytilus*. These authors did not give these little legs any special name, and O. F. Müller (1786) seems to have been the first to use the name cilia, probably from the similar appearance of a group of cilia to eyelashes. The name flagellum seems to have a more recent origin, and is perhaps due to Dujardin (1841), who used the term flagelliform to describe the appearance of cilia on some protozoa.

By 1835 cilia had been found in most of the main animal groups, and the first comprehensive reviews about these organelles were written by Purkinje and Valentin (1835) and Sharpey (1835). To the former authors goes the credit for the discovery of cilia in mammals in 1834. Sharpey described cilia observed by himself and others in protozoa, sponges, coelenterates, ctenophores, turbellarians, rotifers, annelids, molluscs, echinoderms, ectoprocts, tunicates and vertebrates. In addition to reviewing the functions of the cilia in these various groups, Sharpey made some interesting comments on the structure and physiology of cilia. Some authors of that time, including Ehrenberg (1832) and Purkinje and Valentin (1835), seemed to be of the opinion that cilia were moved by small muscles attached to the bulbous base of the cilia, while Grant (1835) suggested that they might move by the flowing of water into and out of a tubular organelle. Sharpey, however, put forward the idea that the cilia actually " contain muscular substance throughout a greater or less part of their length, by which they can be bent or extended". He had indeed noticed that cilia may bend in the main part of their length as well as at the base, although the significance of this observation was not appreciated by some of his contemporaries. The same author observed metachronal waves (although the term metachronal was not used until later), and compared the appearance of these waves to those produced by wind in a corn-field; he explained the waves correctly as being the result of the undulations of the individual cilia composing the waves. Sharpey knew that in some cases cilia were compounded together, and he records that cilia varied considerably in length from $\frac{1}{500}$ in. on the branchiae of *Buccinum* to perhaps less than 0.000075 in. In his review he mentions results of experiments on cilia with electricity, varied temperature, acids, alkalis and various other substances carried out by himself and others, and, although the experiments were not recorded in detail, they demonstrate that the physiology of cilia was already exciting attention.

In spite of this flowering of interest in cilia, little serious work on ciliary activity was carried out until the end of the nineteenth century, when Verworn, Schäfer, Parker and Heidenhain were notable contributors to our knowledge of ciliary functioning, and there was renewed interest in ciliary structure. Much of this earlier work has been summarized by Gray (1928) in his well-known monograph; only the more important of these older works will be mentioned here, while more recent work will be treated in greater detail.

Since the time of Sharpey many workers have added further to our knowledge of the distribution and functions of cilia and flagella. Some idea of the wide occurrence of cilia and flagella may be gained from Table 1. The information given there can only be of a provisional nature in that some records require checking, and in some cases the fine structure of the organelle requires to be examined in the electron microscope to show that it conforms to the standard pattern. This applies particularly to the sensory cilia, which are only recorded as present when there is evidence both that the structures are cilia and that they have a function in the receptor organ, while a question mark indicates that one or other or both of these facts is not established, although the structures are usually regarded as cilia and are reputed to have a sensory function. Cilia or flagella are known to be present in all the major animal groups except the Nematoda, and it is possible that some sensory structures there may reveal their ciliary origin in studies of their fine structure.

The normal function of cilia and flagella is the movement of fluids relative to the attachment of the organelle. If the body bearing the organelle is free to move and is small enough, the movement of cilia or flagella will move the body, otherwise the fluid will be moved over the surface of a stationary body. It would be an easy matter to fill a book of this size with accounts of the ways in which cilia and flagella are used in organisms of all the groups mentioned in Table 1, so that we shall have to be satisfied here with a few general comments of wide application and some reference to useful reviews and other works of value in this context, which are listed in an Appendix on p. 201.

Perhaps it is first necessary to emphasize that the difference between cilia and flagella is a purely functional one. Both structures show an identical structural plan in electron microscope studies, but their mode of use is slightly different. It is characteristic of cilia *sensu stricto* that the resultant movement of fluid caused by their beating is at right angles to the long axis of the cilium when it is at the middle of the active part of the beat (Fig. 1a), and therefore parallel to the surface bearing the cilia. "Typical" flagella, on the other hand, produce a resultant

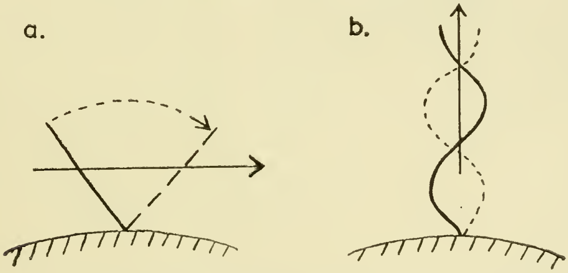


FIG. 1. Diagrammatic representation of the characteristic movement of **a**, a cilium, and **b**, a flagellum. The solid arrow indicates the movement of water and the dotted lines the movement of the organelles.

TABLE 1. THE OCCURRENCE OF CILIA AND FLAGELLA IN VARIOUS SYSTEMS AND ORGANS OF ANIMALS AND PLANTS.

Phylum	Class	Locomotion larval adult	Feeding	Digestive system	Respiratory system	Excretory flame cells nephridia	Sensory	Gametes	Reproductive system	Coelomic circulation	Surface cleaning
Animal kingdom											
Protozoa	Mastigophora	/P	P	/	/	//	P	P	/	/	P
	Rhizopoda	–P	–	/	/	//	/	P	/	/	–
	Sporozoa	—	–	/	/	//	/	P	/	/	–
	Ciliata	PP	P	/	/	//	P	/	/	/	P
Parazoa		P–	P	/	/	//	·	P	/	/	–
Cnidaria		P–	P	P	/	//	P	P	/	/	P
Ctenophora		PP	P	P	/	//	P	P	/	/	P
Platyhelminthes	Turbellaria	PP	–	–	/	P/	?	P	P	/	P
	Trematoda	P–	–	–	/	P/	?	P	P	/	–
	Cestoda	P–	–	/	/	P/	?	P	P	/	–
Nemertina		PP	–	P	/	P/	?	P	–	/	P
Aschelminthes	Rotifera	/P	P	P	/	P/	?	P	P	/	P
	Gastrotricha	/P	P?	–	/	P/	?	?	–?	/	P
	Kinorhyncha	—	–	–	/	P/	?	P	–	/	–
	Priapulida	—	–	–	/	P/	?	?	–	/	–
	Nematoda	—	–	–	/	//	?	–	–	/	–
Acanthocephala		—	–	/	/	P/	·	P	–?	/	–
Entoprocta		P–	P	P	/	P/	?	P	P	/	–
Sipunculoidea		P–	P	P	/	/P	?	P	P	P	–
Annelida		PP	P	P	P	/P	?	P	P	P	P
Mollusca		PP	P	P	P	PP	P	P	P	?	P
Arthropoda		—	–	–	–	$/P^x$	P^z	P^z	P^x	–	–
Ectoprocta		P–	P	P	/	//	?	P	P	P	–
Brachiopoda		P–	P	P	P	/P	?	P	P	P	P
Phoronida		P–	P	P	/	//	·	P	P	–	–
Echinodermata		P–	P	P	P	//	?	P	P	P	P
Pogonophora		P–	P?	/	/	/P	·	P	P	·	?
Chaetognatha		/–	–	P	/	//	?	P	P	P	–
Chordata											
Hemichordata		P–	P	P	P	//	?	P	/	P	P
Urochordata		—	P	P	P	//	?	P	P	–	–
Cephalochordata		—	P	P	P	P/	?	P	/	–	–
Vertebrata		P–	P	P	P	/P	P	P	P	P^y	P
Plant kingdom											
Algae and fungi		P–	/	/	/	//	P	P	/	/	–
Bryophyta, Pteridophyta and Gymnospermae		/–	/	/	/	//	/	P	/	/	–

movement of fluid along the length of the flagellar axis (Fig. 1b), i.e. normal to the surface bearing the flagellum. It is also interesting that a beating flagellum moves fluid continuously, while a cilium is only moving the fluid usefully during part of its beat. In practice, there is no hard-and-fast line to be drawn between the two, for although one can usually see when an organelle is producing a typical ciliary beat, the beat of organelles generally described as flagella may vary from the sine wave pattern shown by some sperm tails, which results from equal and alternate waves of bending on the two sides of the flagellum, to a pattern that is virtually the same as a cilium, in most respects, being asymmetrical and sometimes extremely unilateral. This latter form of beating is common in those protozoa that bear few flagella, but the organelle may show several complete bending waves within its length (see p. 136), which one never finds in true cilia. It is perhaps realistic to regard the flagellum as the original type, which has been specialized in the majority of cases to give the ciliary type of beat.

On the basis of this functional distinction between cilia and flagella, it is interesting that a single cilium would be useless for moving a sperm head, while a flagellum is ideal. Similarly, a few flagella are much more efficient than a few cilia in moving a small protozoon. Large numbers of organelles with a flagellar beat may occur in those cases where they can be used effectively by virtue of their orientation in the functional position. Where many flagella occur on the bodies of some multiflagellate protozoa, their basal structures may be arranged in such a way that the flagellar shafts leave the surface at a fairly acute angle and the flagellar beat can only act to move the body in one direction, so that all the flagella of the body work together to move the organism forward (Pitelka and Schooley, 1958). The same may be true of the multiflagellate sperm of some plants. In sponges the flagellate cells are positioned so that the water currents produced by flagellar beating draw

P, present in at least some members of the group; –, absent; /, system or stage missing in the group; ?, uncertain (for sensory structures, cilia are presumed to be sensory or the sensory structures are presumed to be cilia); ^{x}, in Onychophora; ^{z}, in Insecta; ·, not known; ^{y}, in cerebrospinal cavity and therefore not strictly in the coelom.

Data mostly from Hyman (1939–59) and Grassé (1948–61), with additional information from other references quoted in this book.

water in through the ostia and pass it out through the oscula. Each flagellum causes a current of water to pass in through the palisade of microvilli that makes up the collar of the choanocyte; these microvilli filter food particles from the water current (Fjerdingstad, 1961). Also, in the flame cells found in some invertebrates the flagella run along the tubes in such a direction that their movement attempts to propel fluid towards the external opening. Movement of small bodies and the movement of fluid along tubes can be carried out with at least equal efficiency by cilia, but the ciliary organelles would be arranged with their long axes more or less at right angles to the direction of motion of the fluid.

The widely-held belief that flagella are longer than cilia is certainly not correct. Cilia may be compounded together into large structures that may be much longer than any flagellum. However, there is a mechanical reason why a non-compound organelle with a true ciliary beat is normally short (see p. 172), while a flagellar structure is not limited in length except by factors such as the transport of metabolites to provide energy for contraction. The fact that a structure beats like a cilium does not mean that it must be short, although it is frequently said that flagella are longer than cilia because they show several waves of bending within their length.

Where many of these organelles occur together, they almost always show the ciliary beating pattern; it seems more efficient than the flagellar pattern, and is certainly more adaptable in those cases where a reversal of beat may be required. In such large groups of cilia the continuous even flow of the fluid over the surface is ensured by the rhythmic beating of the cilia which are co-ordinated metachronally in such a way that at any instant there will be some cilia at all phases of their beat; some cilia are in the active phase of beat and others nearby are recovering from their active beat and preparing to perform another. Flagella which occur in groups may beat together, but in their motion it appears that the bending waves travelling down adjacent flagella may be synchronized by mechanical interaction through the water (Pitelka and Schooley, 1958).

The mode of functioning of flagella has been briefly referred to above. Flagella attached to the outside of small bodies like some protozoa and plant spores, or the sperm of animals and some plants, are used to move water from flagellar base to tip, and by their action they move the body forwards either directly (Pl. XV,) or indirectly by causing a gyration and rotation of the body (Fig. 34, p. 135). Flagella inside the body generally seem to function in moving fluids along channels as in the sponge or flame cells (Fig. 2a, b). Here the flagella lie in the lumen of the channel and move water from base to tip. Flagella have been described in other places in the bodies of animals, but their motion has not been thoroughly studied.

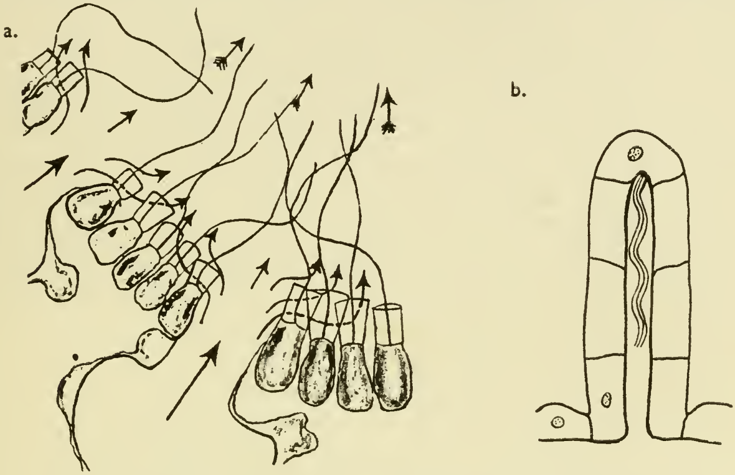


Fig. 2. **a,** The arrangement of flagella and the water movements that they cause in a sponge (from Bidder, 1923). **b,** Diagram of a flame cell system showing the position of the flagella.

Cilia may have mechanical functions in moving fluids over open surfaces and in tubes. In both situations there may be cilia whose function is to move a shallow film of fluid over the cell surface, or, alternatively, the cilia may be responsible for more distant currents in the fluid. Generally, those cilia which produce these more distant currents are longer and larger (frequently compound) and are able to sweep through a larger volume of water than the cilia which move the surface film.

The movement of a thin film of fluid is perhaps the more common function of cilia, for in animals of most groups mucous strands are transported by ciliated epithelia. The moving strands of mucus may carry entangled debris where the cilia are performing a cleansing function, or may also carry food particles in the case of cilia used in feeding and in some digestive systems. Under this heading also come the locomotory cilia of some forms, such as the unspecialized somatic cilia of some protozoa and some larval forms, and the locomotory cilia of some nemertines, molluscs, turbellarians and gastrotrichs. In ciliated tubes where the diameter of the tube is large compared with the cilium length, the cilia may function in a similar way, with either a cleansing function or a special transport function, e.g. in the respiratory and female reproductive tracts of mammals. Where the tube diameter is narrow the whole of the fluid contents of the tube may be moved.

Cilia which create currents at a greater distance from the surface generally have a function in locomotion, feeding or respiration. The compound cilia that make up the comb-plates of ctenophores are perhaps the extreme example of locomotory cilia; their size is such that they move through a large body of water, and a succession of plates beat the water like a paddle-wheel. Many of the more specialized larval forms like the molluscan

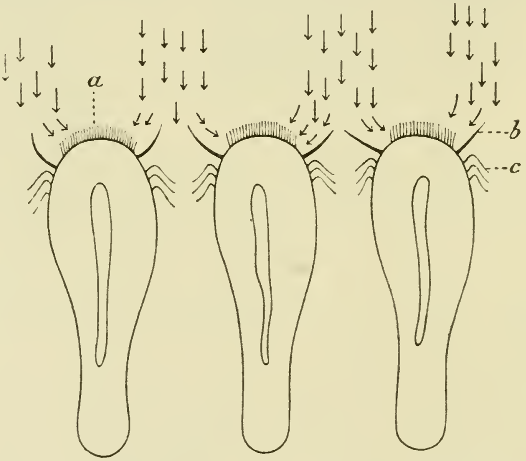


FIG. 3. The arrangement of cilia on the gill filament of *Mytilus* and some of the water movements that they create (from Gray, 1928).

veliger, as well as rotifers and some ciliated protozoa, use large cilia for locomotion. Good examples of cilia whose primary function is the collection of food are found in the lamellibranch molluscs where large currents of water are passed between the ctenidial filaments by the action of the lateral cilia, while other cilia on the filaments serve to extract the food particles from the water and carry them away (Fig. 3). Very similar arrangements are found in the " gill " structures of brachiopods, ascidians and cephalochordates, while some annelids, ectoprocts and entoprocts use ciliated tentacles, which look remarkably like ctenidial filaments in transverse sections, for the same function. The typical rotifers and many protozoa carry their feeding cilia on less specialized structures, and yet produce very considerable feeding currents. Very often the currents produced by these cilia must also satisfy the respiratory needs of the animal, but in others cases special respiratory currents are required, e.g. on the parapodia and " gills " of many polycheate worms, especially in tubicolous types where very large cilia may be present.

It was pointed out by Gray (1928) that the movement of fluids in tubes by cilia will only be reasonably effective if the radius of the tube is not more than 4 or 5 times the length of the cilia. In larger tubes there will be a central region in which the fluid moves much less rapidly than near the walls of the tube, so that in tubes of very large diameter only a relatively thin film of fluid at the tube surface is in effective motion. In the case of nephridial tubules or the male reproductive ducts of many animals, the diameter is small and cilia can effectively move all the fluid in the tube.

Sensory structures whose organization is based upon a typical ciliary fibril pattern are known from a variety of animal groups and it is likely that many more will be discovered as more receptor structures are studied with the electron microscope. Several of these structures are described on pp. 32-35. In some protozoa cilia have been modified for supporting functions, but their ciliary origin may be inferred from the arrangement of internal fibrils, at least at the base (see p. 36).

The functions that cilia can perform are taken over by muscles in the majority of animals. Thus, fluids are moved by muscular contraction in circulatory systems of many animals, while the limbs of some crustaceans are used to create feeding currents from which food particles can be filtered. Many endoparasitic forms show a loss of at least the external cilia, and often many internal cilia also; it is understandable that many gut parasites particularly should produce a cuticle for protection. It is also important that cilia cannot function in air, while muscles can be used to move air and can function to move structures in air. Although it appears that there are no biological functions which can only be performed by cilia, except perhaps for the cleansing of the surfaces of living cells, many animals which rely mainly on muscles have retained cilia for some purposes.

CHAPTER 2

THE STRUCTURE OF CILIA

1. Introduction

The observations of earlier workers using the light microscope led them to put forward a variety of opinions concerning the structure of cilia and their bases. Among these workers were those who believed that sperm tails (Ballowitz, 1888) and cilia (Koltzoff, 1903) had an organization of internal filaments. The similarity of ciliary bases to centrosomes was noted by Henneguy (1898) and von Lenhossek (1898), who in fact suggested that the basal bodies of cilia were centrosomes, an idea that has since been extended, especially by protozoologists. Many studies of the protozoa (e.g. Schuberg, 1891; Maier, 1903; Sharp, 1914), as well as some studies of metazoa (e.g. Grave and Schmitt, 1925), indicated the presence in the cytoplasm of fibrils connected to the ciliary basal bodies. Many of these early observations involved objects so near the limits of resolution of the light microscope that it is not surprising that they did not always agree; indeed, in many cases it is surprising that the observations could be made at all.

The use of the electron microscope allows more detailed and accurate information to be obtained about the structure of cilia. From a large number of electron microscope studies it is evident that the shafts of cilia, flagella, sperm tails and certain structures derived from these have a common structural plan. Earlier electron micrographs showed that cilia fray into a number of longitudinal fibrils; there were usually eleven of these fibrils, two of which were rather different from the other nine. The thin-sectioning technique was used to show that the fibrils are arranged in a particular way, with a ring of nine fibrils around two at the centre, and that the whole bundle (axoneme) is surrounded by a membrane that is continuous with the cell membrane. Similarly, the structure of the basal body seems to be consistent, appearing in sections as an empty cylinder whose walls contain continuations of the nine peripheral fibrils of the shaft, while the two central fibrils of the ciliary shaft end at about the level of the cell surface. In many cases fibrillar structures are attached to these basal bodies and may be connected to other structures of various sorts including the basal bodies of other cilia.

This basic pattern is not entirely consistent, however, for important differences occur. The structural pattern of cilia remains fairly constant, functional differences depending on their co-ordinated action and sometimes on their association into compound structures. The flagellar pattern is more widely modified in protozoan and algal flagella as well as in sperm tails.

The most recent electron microscope work with improved techniques indicates that the basic pattern is even more complicated than was believed a very few years ago. These reports are so consistent in some details that it seems reasonable to base a description of " a typical cilium " upon a few of the more recent observations and follow this with an account of the variations from this basic pattern.

It should not be imagined that we have reached the limits of magnification of biological material with the electron microscope. Theoretically the electron microscope can be used to examine structures down to about 1 Å, and instruments which can resolve objects smaller than 10 Å are in use, but the limit for good resolution in biological material is at present in the region of 30 Å, or sometimes 20 Å, although few workers have achieved this. As methods of fixation, ultra-thin sectioning and " electron staining " improve, it should be possible to approach more nearly to the theoretical limit. We can therefore expect the electron microscope to reveal yet more of the details of cilium structure as techniques are refined.

The structure and functioning of cilia and flagella are so similar that one description will hold good for both organelles in most respects. A term descriptive of both cilia and flagella is really required, but, since none is in common usage, the organelle will be referred to as a cilium in general descriptions, and the term flagellum will be used only for structures which are believed to have a flagellar beat.

2. The Structure of a Typical Ciliary Organelle

Shape and Size

Most cilia have a constant diameter from the base to within a short distance of the tip, where there is a gradual taper to a blunt point. Some cilia show a greater or lesser length of thinner whiplash region at the end of the cilium, e.g. the somatic cilia of *Stentor* (Pl. VII h) (Randall and Jackson, 1958), or the posterior flagellum of spermatozoids of *Himanthalia* (Manton, Clarke and Greenwood, 1953). The special shape of some sperm tails is caused by sheaths of mitochondria and other thickenings around the main axis.

The diameter of ciliary organelles, which is more constant than the length, usually lies between 0·15 and 0·3 μ, although some sperm tails and flagella with additional structural components may have a diameter larger than 1 μ. Single cilia commonly have a length of 5 to 20 μ, but where they are combined into compound structures they may be very much longer, e.g. up to 2000 μ in the ctenophore *Mnemiopsis* (Afzelius, 1961a). Flagella have been found to have lengths of the order of 5 to 150 μ, while sperm tails tend to be longer, up to 200 μ or more. It seems that the dimensions of ciliary organelles must be closely bound up with their functioning (see p. 172). The lengths and diameters of some cilia, flagella and sperm tails are quoted in Table 2.

The Shaft

The **membrane** which completely encloses the cilium is continuous with the plasma membrane of the cell. It often appears wrinkled in fixed material, but in life it is probably smooth except in special cases like compound cilia and " flimmer-flagella," where it bears surface appendages. From high-resolution electron micrographs it appears that this membrane is three-layered, with a dense layer 20 to 30 Å thick on either side of a less dense 30 Å layer, giving a total thickness of about 70 to 90 Å.

The two **central fibrils,** which run parallel for the length of the shaft of the cilium, occupy a central position with about 300 to 350 Å between their centres, as shown in Fig. 4b. In transverse section they appear tubular with a total diameter of 150 to 250 Å (see Table 3), and a wall thickness of 40 to 50 Å. It appears from

TABLE 2. THE DIMENSIONS OF SOME CILIA AND FLAGELLA

Organism and part of body	Organelle	Length(μ)	Diameter (μ)	Reference
Micromonas (*Chromulina*) *pusilla*	Flagellum	3–4	0·15[a]	Manton (1959a)
Chrysochromulina spp.	Flagella	10–40	0·25–0·3	Parke, Manton and Clarke (1955-9)
Pteridium (spermatozoid)	Cilium (?)	15	0·2	Manton (1959b)
Cycas/Zamia (spermatozoids)	Cilia (?)	35	0·2	Barton (1962)
Peranema (anterior of two)	Flagellum	100	1·0[b]	Roth (1959)
Trichonympha	Flagellum	20–150	0·25	Gibbons and Grimstone (1960)
Paramecium	Cilium	10–12	0·27	Sedar and Porter (1955)
Mnemiopsis (comb-plate)	Cilium[c]	2000	0·3	Afzelius (1961a)
Rattus (trachea)	Cilium	5	0·24	Rhodin and Dalhamn (1956)
Mus (retinal rod)	Cilium[d]	0·7	0·16–0·24	De Robertis (1956a)
Psammechinus	Sperm tail	40–45	0·21[e]	Afzelius (1959); Gray (1955)
Perameles	Sperm tail	200	1·5–1·7[f]	Cleland and Rothschild (1959)
Homo (entire tail)	Sperm tail	55		Ånberg (1957)
(mid-piece)		4–5	1·0[g]	
(principal-piece)		45	0·3–0·4[f]	
(end-piece)		5–7	0·18[e]	

[a], distal ⅔ of flagellum narrower; [b], diameter includes intraflagellar material and sheath; [c], unit of a compound cilium; [d], modified connecting cilium of sensory cell; [e], no sheath present; [f], diameter of principal-piece with fibrous or spiral sheath; [g], with mitochondrial sheath.

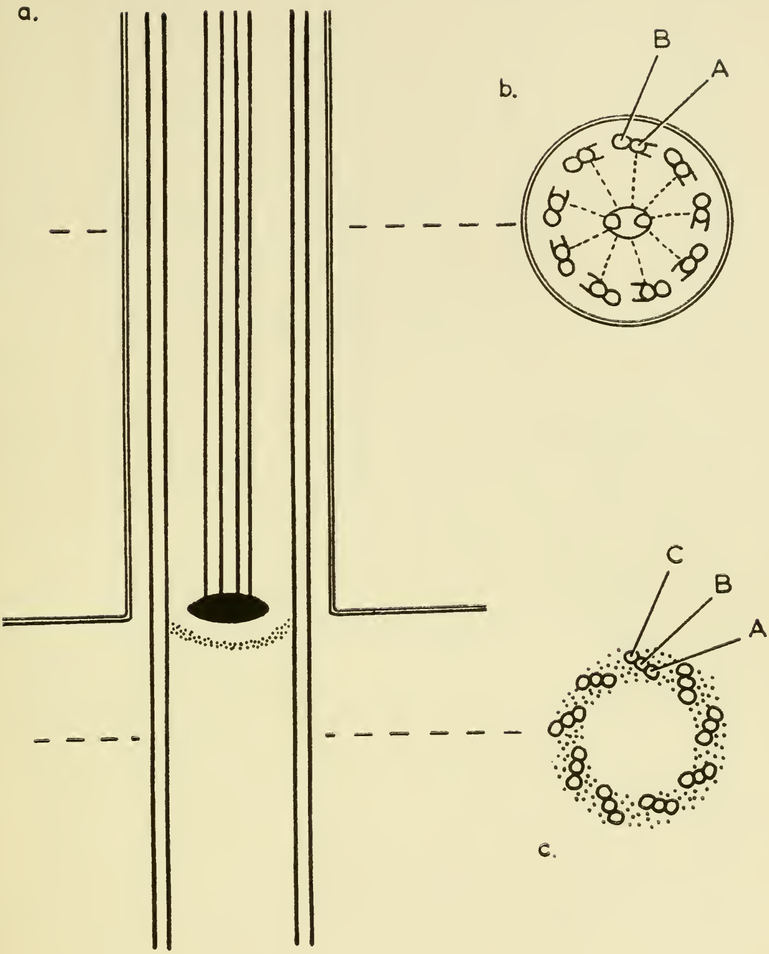


FIG. 4. Diagrams showing the structure of a cilium. **a,** Structures seen in a longitudinal section. **b,** Transverse section of the ciliary shaft with the typical arrangement of internal fibrils. **c,** The arrangement of fibril triplets in the basal body, showing the position of the third subfibril C, and the twist of the peripheral triplets. Compare with Plates I to IV.

TABLE 3. SOME EXAMPLES OF THE DIMENSIONS OF THE AXIAL FIBRE BUNDLE AND ITS COMPONENT FIBRILS

Organism	Organelle	Diameter of fibre bundle (μ)	Diameter of central fibrils (Å)	Diameter of peripheral fibrils (Å)	Reference
Trichonympha	Flagellum	0·16–0·17	240	250 × 370	Gibbons and Grimstone (1960)
Paramecium	Cilium	0·2	150–200	150–200 × 300–400	Sedar and Porter (1955)
Anodonta	Cilium	0·2	240	260 × 380	Gibbons (1961b)
Cycas/Zamia (sperm)	Cilium ?	0·15	200	200 × 330	Barton (1962)
Rattus (trachea)	Cilium	0·16	175–215	235 × 330	Rhodin and Dalhamn (1956)
Mus (retinal rod)	Cilium	0·16	absent	160[a]	De Robertis (1956a)
Perameles	Sperm tail	0·15	200–250	250–300[a]	Cleland and Rothschild (1959)
Homo	Sperm tail	0·15	210	190 × 320	Ånberg (1957)

[a], doublets of peripheral fibrils were not found by these authors.

longitudinal sections that these central fibrils normally run straight from one end of the cilium to the other without spiralling, a finding that is corroborated by transverse sections in those cases where a reference point is available in a series of sections, e.g. in compound cilia of known shape. The presence of two central fibrils confers a degree of bilateral symmetry on the ciliary axis (see p. 20 for further discussion). Cross-striations with a periodicity of 130 Å, probably caused by spiral thickenings, were found in the central fibrils of *Pseudotrichonympha* by Gibbons and Grimstone (1960) and in *Anodonta* by Gibbons (1961b).

In many transverse sections at high resolution some additional central structure is visible. This takes various forms, but the most commonly described is a membrane or sheath in the form of a central tube surrounding or linking the two central fibrils (Fig. 4b). Sometimes this sheath has been found to have a spiral structure (Manton, 1956; Gibbons and Grimstone, 1960; Gibbons, 1961b). It is believed that this sheath around the pair of central fibrils could give rise to the appearance of a quartet of central fibrils that is seen in a number of electron micrographs, e.g. Manton (1957) and Grassé (1956). One or two further direct links were found between the two central fibrils in *Anodonta* cilia by Gibbons (1961b), as shown in Fig. 5a. An additional " mid-filament " (see Fig. 5) has been found by Afzelius (1961a) in *Mnemiopsis* cilia and by Gibbons (1961b) in *Anodonta* cilia; in the latter it appears in transverse sections as a dense dot some 50 Å in diameter, but it is not quite as clearly defined in *Mnemiopsis* cilia where a structure about 100 by 180 Å was found in transverse sections. Neither structure has been seen in longitudinal sections, so that there is no evidence that it is a longitudinal filament, although it consistently appears in a particular position in transverse sections of the cilia. Incidentally, the mid-filament was found to be nearest to peripheral fibril 1 in *Anodonta* and nearest to peripheral fibrils 5 and 6 in *Mnemiopsis*, and it is interesting that the normal direction of the effective stroke of the ciliary beat is away from the side on which the mid-filament occurs in both species, i.e. the effective stroke is towards fibril 1 in *Mnemiopsis* and towards fibrils 5 and 6 in *Anodonta*.

Around the central fibrils the nine longitudinal **peripheral fibrils** are arranged to form a cylinder of outside diameter about

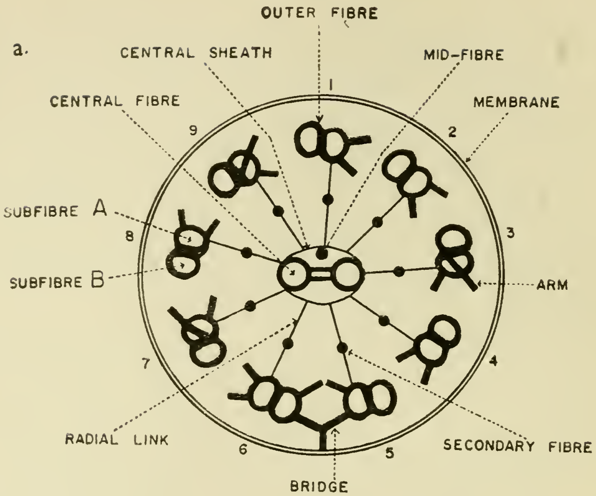


FIG. 5. Details of structure seen in transverse sections of ciliary shafts (see text). **a**, Diagrammatic transverse section of a cilium of *Anodonta* (from Gibbons, 1961b).

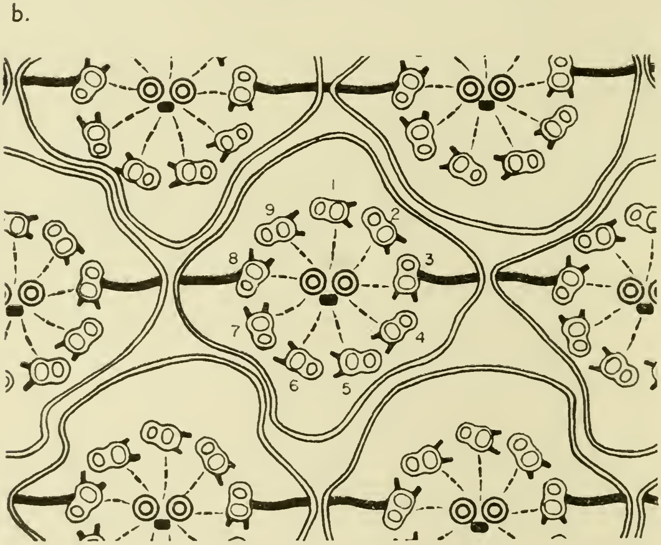


FIG. 5b. Diagram of a cilium from a comb-plate of *Mnemiopsis*, showing the way in which cilia are packed together (from Afzelius, 1960).

1600 Å. Each of these fibrils is double, as shown in Figs. 4b and 5, being composed of two subfibrils which appear in transverse sections to be tubular, with a wall about 60 Å thick and a circular or elliptical outline of diameter about 200 to 250 Å (see Table 3). The dividing wall between the two subfibrils is also about 60 Å thick. Recent electron micrographs, particularly those of Afzelius (1959, 1961a) (Pl. Xa and Vb), Gibbons and Grimstone (1960) (Pl. 1) and Gibbons (1961b) (Pl. IId), have shown the presence of two longitudinal rows of " arms " on one of the subfibrils (labelled A in the figures) of each peripheral doublet. These arms can be seen in both transverse and longitudinal sections (Fig. 7) as rods about 120 to 150 Å long and 50 Å thick, which are spaced about 130 Å between centres in longitudinal rows, and which project perpendicular to the axis of the shaft towards the adjacent peripheral doublet. Gibbons (1961a) has found that in all the cases he examined the arms project in a clockwise direction, as seen by an observer looking from the base. Subfibril A, which bears the arms, often has a denser core than the other subfibril; in at least some cases this is because one of the arms extends back across the subfibril, as seen in Fig. 5a. This subfibril has sometimes been found to be larger than subfibril B (Fig. 5b). In echinoderm sperm tails (Afzelius, 1959) and *Anodonta* cilia (Gibbons, 1961b), arms are present on subfibril B of peripheral fibril number 6; these link with those of the A subfibril on fibril 5 to form a complex bridge, from which a link runs to the ciliary membrane in *Anodonta* cilia (Fig. 5a). Gibbons and Grimstone (1960) and Lansing and Lamy (1961a) have found some indications of a helical substructure in these peripheral fibrils; the latter authors found a helical strand 40 Å thick with a 140 Å pitch, which may be associated with the arms.

Bradfield (1955) postulated that the nine peripheral fibrils are spaced at equal distances from adjacent fibrils and also at equal distances from the centre of the cilium. He also suggested that the fibril arrangement is symmetrical about a line at right angles to the line through the central fibrils, as shown in Fig. 6a. Most authors have agreed with these hypotheses, but first Cleland and Rothschild (1959) and then Lansing and Lamy (1961a) have challenged both of them. These two pairs of workers have found that the fibrils were not equally spaced from their neighbours, some spacings being wider than others, e.g. see Fig. 6c. Measurements made by the author on the best micrographs of transverse sections of cilia and flagella published by a number of workers have shown that such an inequality does occur, but they also show that in all cases the ring of fibrils is not a true circle. This is likely to result from distortion in the preparation of the sections, and an example is shown in Fig. 6b. Peachey (1958) has shown that 30 to 50 per cent of " compression " may occur in thin sectioning for the electron microscope, and much of this may remain after flattening of the sections. In all the cases examined the inequality of spacing was regularly distributed around the peripheral ring in a manner that would be expected from such distortion. Until we can be sure of obtaining undistorted sections, or of knowing accurately the effects of compression, this point will not be proved beyond doubt.

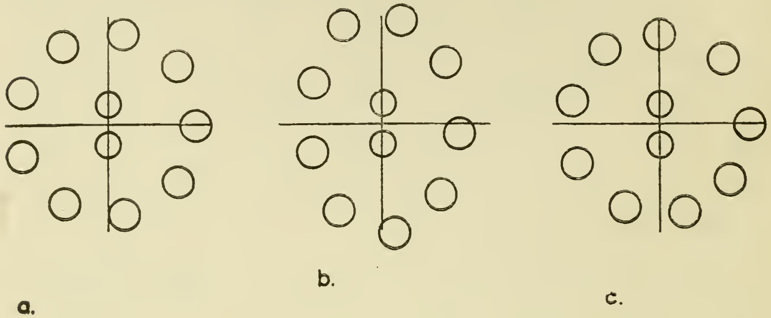


FIG. 6. Diagrams of the distribution of fibril doublets in the axial bundle of cilia (see text).

The same distortion makes it difficult to be certain of the symmetry of fibril arrangement. According to Bradfield's hypothesis, one fibril of the peripheral ring is bisected by the plane of symmetry (at right angles to the plane through the two central fibrils), and this fibril should therefore be distinguishable from the others. This idea has been used as a basis for numbering the fibrils of the peripheral ring (see Fig. 5), taking the fibril in the plane of symmetry as number 1, and then numbering the remainder in a clockwise direction, i.e. in the direction in which the arms point (Afzelius, 1959). This numbering system will be used in this book as a convenience, although, as Gibbons and Grimstone (1960) have found, it is not always possible to identify fibril 1 with certainty, so that these authors were doubtful about the value of such a numbering system at present. Cleland and Rothschild (1959) and Lansing and Lamy (1961a) found that frequently one fibril was situated in the plane joining the two central fibrils, but this has not been widely observed, and once again distortion may be the cause. Alternatively, it is possible that one subfibril of each pair is more important than the other in determining the distribution of fibrils, e.g. the A subfibrils are connected to the central structures by radial strands (see below), and may be symmetrically arranged with respect to the plane through the central fibrils. Many observations, including the arrangement and size distribution of additional fibrils and sheath thickenings in sperm tails (see pp. 43–48), the position of the compartmenting lamellae of ctenophore comb-plate cilia (see Fig. 5b and p. 32) and the bridge structure between the fibrils 5 and 6 in *Anodonta* cilia (Fig. 5a), can be used as evidence that there is a plane of bilateral symmetry which passes through the fibril number 1 in the majority of ciliary organelles. Superimposed on this is of course the asymmetry resulting from the presence of the arms on the subfibrils, as stressed by Gibbons (1961a).

In earlier studies the **matrix** enclosed by the membrane and surrounding the fibrils was thought to be structureless and of similar density to the cell cytoplasm. Local regions of increased density have been seen in many recent studies. Most of these are best explained as radial spokes connecting the armed subfibrils of each peripheral doublet with the central fibrils (Figs. 4b and 5). Gibbons and Grimstone (1960) found their observations on *Pseudotrichonympha* to be consistent with the suggestion that a ring of nine longitudinal secondary filaments about 50 Å in diameter was present half-way between the central and peripheral fibrils; these may be connexions between the radial links. In *Anodonta* cilia Gibbons (1961b) has found that both radial links and longitudinal filaments are again present, although the longitudinal continuity of the secondary filaments is not established. Radial strands occur at 270 Å intervals in *Anodonta* (i.e. at twice the distance between the arms) and at 400 Å in a trichonymphid flagellate (i.e. at three times the distance between the arms) (Gibbons, 1960). Additional structures have been found in the matrix in some special cases and will be mentioned later.

The structure of **the tip of the shaft** of cilia is usually difficult to study. In micrographs of frayed whole cilia it was found that the longitudinal fibrils are not all of the same length, so that the tapered tip of the cilium could be caused by the fibrils coming to an end at different distances from the tip (e.g. Bradfield, 1953). The series of transverse sections at different distances from the tip of *Pseudotrichonympha* flagella reproduced in Plate IIIa shows that fibrils first lose the arms, then one subfibril of each doublet is lost; at this level the fibril spacing is a little less regular and the peripheral ring has a rather smaller diameter (Gibbons and Grimstone, 1960). The single fibrils then terminate one by one, but it is uncertain in *Pseudotrichonympha* whether the central fibrils run to the tip or not.

Rather similar changes occur at the tips of cilia of *Anodonta* (Gibbons, 1961b), where the arms, secondary filaments and radial links of any fibril all disappear at a particular level, although the levels for different fibrils may be slightly different. When the radial links and arms disappear the peripheral fibrils lose their regular arrangement.

In *Euplotes*, Roth (1956) found that the central fibrils were the longest, while Manton and Clark (1951) found that the central fibrils were the shortest in cilia of the spermatozoid of the fern *Dryopteris*. Several arrangements were found at the tip of sea urchin sperm tails by Afzelius (1959); sometimes the central fibrils disappeared and the peripheral fibrils were reduced in number while still double, and in other cases the peripherals were reduced to single fibrils before the central fibrils disappeared. No support has been forthcoming for the finding in rat tracheal cilia by Rhodin and Dalhamn (1956) that the peripheral fibrils meet and fuse at the tip. In all cases the diameter of the cilium gradually decreases towards the tip until transverse sections are obtained which appear solid, so that the end of the cilium is closed.

The Transition from Shaft to Basal Body

In the ciliary basal body the central fibrils are found to be lacking while the walls of the cylindrical structure contain continuations of the nine peripheral fibrils, usually in triplets rather than doublets (see Fig. 4c).

The central fibrils come to an end at the level of the cell surface

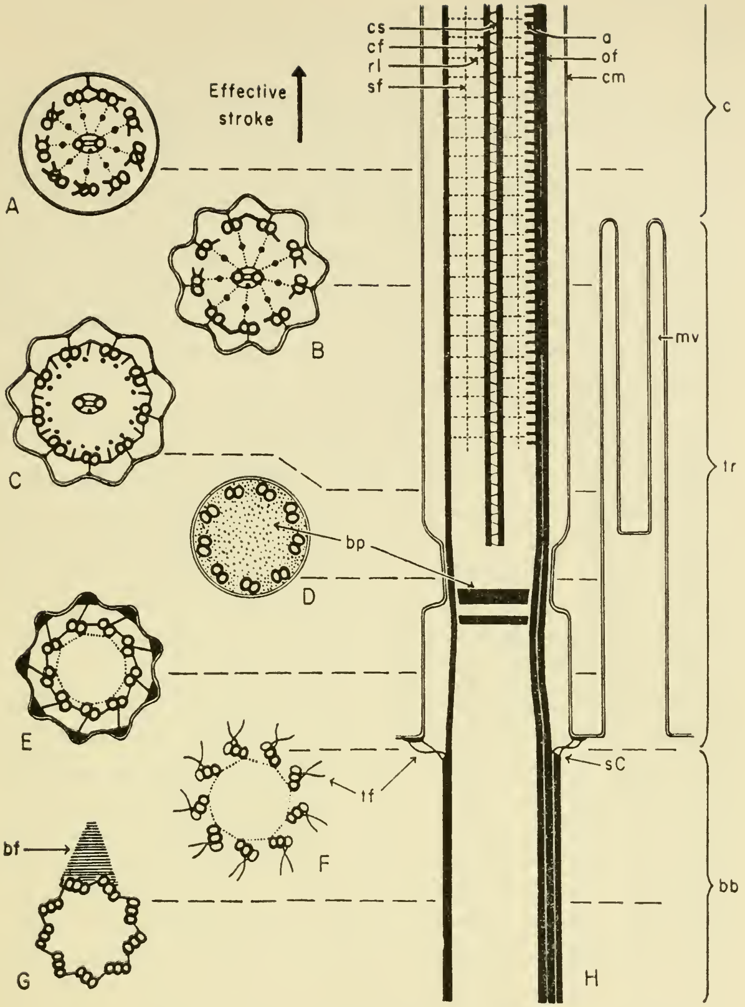


FIG. 7. Diagrammatic reconstruction of the proximal part of a cilium, the transition region and the basal body (*Anodonta*).

A to **G** show transverse sections at the levels indicated; they are all orientated with the plane of beat up and down in the figure, with the effective stroke towards the top. **H** shows a median longitudinal section in the plane of the central fibrils; the outer fibril on the right is turned sideways to show it more clearly (from Gibbons, 1961b).

a, arm; *bb*, basal body; *bf*, basal foot; *bp*, basal plate; *c*, ciliary shaft; *cf*, central fibril; *cm*, ciliary membrane; *cs*, central sheath; *mv*, microvillus of brush border; *of*, outer fibril; *rl*, radial link; *sC*, distal end of subfibril C; *sf*, secondary filament; *tf*, transitional filament; *tr*, transitional region of the cilium.

(Pl. I, II and IIIb), or a little above it, where there is usually a transverse membrane or plate (Figs. 4 and 7). This appears to extend the whole width of the cilium in at least some cases, and is often associated with a neck-like narrowing of the ciliary shaft for a short distance (Fig. 7H and Pl. IIa). Above this membrane there is usually a spherical or discoidal granule (the axosome) about 800 Å in diameter at which the central fibrils end (Pl. IVb), while one of the central fibrils of *Pseudotrichonympha* ends at a crescentic body which is fused to some of the peripheral fibrils at the level of the transverse membrane.

The transition from peripheral doublets in the shaft into triplets in the base was first thoroughly worked out in *Pseudotrichonympha* by Gibbons and Grimstone (1960). In a transverse section of the shaft, the line joining the centres of the two subfibrils of any peripheral doublet is tilted at about 5° to the tangent to the circle of peripherals, with subfibril A slightly closer to the centre of the shaft. No arms are present on fibrils in the transition region or immediately above it, but a third subfibril appears at the transition beside each of the nine doublets which are continued into the base from the shaft. Afzelius (1959) suggested that the third subfibril replaces the arms, but Gibbons and Grimstone found that the third subfibril appears on the opposite side of the armless subfibril (i.e. in position C), and so cannot be a continuation of the arms. The three subfibrils of each triplet appear tubular and about the same size as the subfibrils of the doublets in the shaft. Over a distance of about 1 μ around the transition region each peripheral fibril twists, so that in the basal body the line joining the centres of the three subfibrils of any triplet makes an angle of about 45° with the tangent to the circle of triplets (Pl. I). According to Gibbons (1961a), each triplet is inclined inwards in a clockwise direction as seen by an observer looking outwards from the inner end of the basal body.

The details of a rather more complex transition zone, which has been found in cilia of *Anodonta* by Gibbons (1961b), are illustrated in Fig. 7. Gibbons considers that the basal body proper is that region in which the peripheral fibrils are triplets, and that the region of the cilium between the distal end of subfibril C and the proximal end of the typical shaft structure is the transition zone. This transition zone is about 0·8 μ long in *Anodonta*. The changes taking place in the transition zone will be described as they appear in transverse sections at successively lower levels of the cilium, i.e. as in diagrams A to G of Fig. 7.

The first change at the outer end of the transition zone is the appearance of links between the peripheral fibrils and the ciliary membrane (Fig. 7B). At the same level some of the arms may become less obvious and an additional bridge-link is developed between fibrils 1 and 2. This structure continues for about half of the length of the transition zone before an abrupt ending of radial links and secondary filaments, and the appearance of similar bridge-links between all of the peripheral fibrils (Fig. 7C). In addition, a ring of 18 (probably) dense dots appears just inside the ring of peripheral fibrils. A little below this the two central fibrils and associated structures end, and the diameter of the ciliary membrane is decreased to form a tight collar around the axial bundle of fibrils (Fig. 7H). A pair of plates run across the cilium at this level and separate the contents of the shaft from the basal body cavity; they form a fairly dense region around the peripheral fibrils. Below these basal plates the diameter of the ciliary membrane increases once more, and the peripheral doublets, which retain connexions to the membrane and to each other, are shown to be tilted further (Fig. 7E). Connexions between all the A subfibrils may develop and the A-B connexions disappear before the inner end of the transition region is reached. The C subfibrils appear at about the level of the cell surface; at their distal end each one is associated with two fine transitional filaments, which run to the cell membrane (Figs. 7F and 7H). The triplets of the basal body run inwards from this level and are now tilted at an angle of about 40° to the tangent to the ring of fibrils.

Gibbons points out that the significance of this complexity is obscure, as is also the connexion between the structures found at the different levels. He stresses the problems of the development of such a complex structure by outgrowth from the cell. The transition zone in *Anodonta* occupies the thickness of the brush border of the cells; the presence of the brush border may influence the development of the cilia.

It is not known whether other cilia have complex transition zones like these, but we have no reason to doubt it. Further studies may reveal a variety of individual patterns or some common features of structure. The transition zone is obviously an important part of the cilium, for it links the basal region, responsible for excitation and anchorage, to the contractile shaft. It is interesting that connexions between the peripheral fibrils and the ciliary membrane in the transition zone have been widely found, and these may always occur, e.g. in the connecting cilium of the mammalian retinal rod (Tokuyasu and Yamada, 1959) and the sperm of *Hydroides* (Colwin and Colwin, 1961), as well as in some cilia and flagella mentioned above.

The Basal Body.

The kinetosome or basal granule of ciliated protozoa, the the blepharoplast (or only part of this, according to some authors) in flagellated protozoa, and the centriole, or one of the centrioles, at the base of the flagellum of sperm, are the basal bodies of the ciliary organelles that these cells carry. The similarity of the basal body to the centriole present in many animal cells, and the derivation of the basal body from the centriole will be discussed later (see p. 48).

The basal body is a cylindrical structure, usually 4000 to 5000Å long and 1500 to 2000 Å in diameter (see Table 4, p. 53) with a less dense central region and a dense wall containing the continuations of the peripheral fibrils of the shaft together with intercalary matter which links the fibrils into a continuous cylinder. The outer end of the basal body is continuous with the ciliary shaft, while the inner end may be open and continuous with the cell cytoplasm, or closed by opaque material which is usually connected to some sort of root structure. Fibres in the cell cytoplasm may be connected to any part of the basal body, most frequently at the inner end, and quite often also near the middle; in both places the wall of the cylinder may be thickened at the fibre attachments (see Pl. IVb).

The central region of the basal body is seldom completely structureless. Granules about 200 Å in diameter have often been observed and are thought to have a connexion with the reputed duplication and fibril production properties of the basal body. Randall and Jackson (1958) report that kinetosomes of *Stentor* are stained by the Feulgen technique, and that the internal granules are most frequent in mitotic or conjugating animals, while

Rouiller and Fauré-Fremiet (1958) found that granules present in the resting (shaftless) kinetosomes of the peritrich ciliate *Ophrydium* disappear on development of the ciliary shaft. Larger structures of a variety of shapes have been observed in the centres of basal bodies; sometimes they are amorphous masses, and sometimes fibres like those in *Euplotes* cilia (shown in Pl. IVb) that Roth (1956) believes to be continuous with the central fibrils of the shaft. A complex cartwheel structure of radiating fibres has been found in *Pseudotrichonympha* and *Trichonympha* (see p. 55 and Pl. I) and also in *Paramecium* Gibbons (1960) and the trypanosome *Blastocrithidia* (Vickerman, 1962).

The peripheral fibrils, which appear as triplets of tubular subfibrils in the basal body wall in the best resolved transverse sections, can usually be traced through to the inner end of the basal body. In some cases, e.g. rat tracheal cilia (Rhodin and Dalhamn, 1956), the subfibrils become less regularly arranged towards the inner end and some diverge and continue into the cytoplasm as short rootlets. More frequently the fibrils continue in their ordered arrangement to the inner end of the basal body, where they terminate at more or less the same level. Lateral inter-connexions between adjacent triplets may be quite distinct, as in *Pseudotrichonympha* (Plate I), where the main connexions are about 45 Å thick, though it is uncertain whether they are fibrils or longitudinal laminae. More usually there is a thick annular zone of dense material in which the peripheral fibrils are embedded to form the basal body cylinder; the use of improved techniques may allow this zone to be further resolved in these cases.

Ciliary Roots

Rootlet structures which make connexion with the basal bodies, and run in the cell cytoplasm, form the most variable part of the ciliary structure, but fall into three main categories. Firstly, there are fine fibrils about 150 to 300 Å in diameter with a tubular appearance in transverse section, which are frequently aggregated into bundles (see e.g. Pl. IVa, Va, XIIIb). These fibrils are usually " connected " to some other structure in the cell, often the basal bodies of other cilia or similar fibrils from other basal bodies, but sometimes they appear to end blindly in the cytoplasm.

Fibrils of the same size and appearance are found in various parts of many cells, and particularly in protozoa. Secondly, fine fibrils may be found connecting the basal bodies of the component cilia of compound structures like cirri and membranelles (see Pl. Va) Similar fine connexions (about 20 Å in diameter) have been found between adjacent basal bodies of *Trichonympha* flagella (Fig. 17, p. 72).

The third type of root takes the form of striated fibres. The diameter of these fibres varies between wide limits and the striation period usually lies between 300 and 700 Å, with or without intraperiod bandings. In fact, several of the best resolved micrographs of fibres show a period of 650 to 700 Å with several intraperiod bands (see Pl. XIV) (Fawcett, 1958b; Gray, 1960; Anderson and Beams, 1959), comparable with the 550 Å periodicity of discharged trichocysts of *Paramecium* (Sedar and Porter, 1955) and the 640 Å periodicity of collagen fibres, although collagen is normally found extracellularly and has a somewhat different intraperiod banding pattern. Several workers have found the striated fibres to be made up of many longitudinal filaments; these were 50 Å in diameter in the rotifer *Philodina* (Lansing and Lamy, 1961a) and 45 Å in diameter in *Anodonta* (Gibbons, 1961b) (Pl. XIVc). Striated fibres may connect or form bundles with those from other cilia as do the striated kinetodesmata of some ciliated protozoa, or may run deep into the cytoplasm of the cell without any obvious connexion as do the roots of the cilia of the locust scolopale organ (Gray, 1960) and some cilia of molluscs and other metazoa (Fawcett, 1958b).

Chemical Nature

We are sadly ignorant about the chemical nature of the parts of cilia and flagella. The recent work of Tibbs (1957) on flagella of the alga *Polytoma* and on fish sperm tails, and of Child (1958, 1959) and Watson, Hopkins and Randall (1961) on cilia of the protozoon *Tetrahymena* have confirmed some previous suggestions and also brought out some new facts. The first problem is to isolate the ciliary organelles and make certain how much has been lost in the preparation. Child found by electron microscopy that his isolated cilia were bundles of fibrils, but could not be certain how many fibrils were present in the bundle, or if the membrane was present;

Watson *et al.* showed that both the membrane and the full complement of fibrils were present in their preparation of isolated cilia. Tibbs seems satisfied that the membrane and matrix contribute little to the dry mass of the organelles.

All authors found that most of the material of the cilium was protein, although lipids, which probably came from the sheaths, form an appreciable part of fish sperm tails. This second observation confirms the findings of Miescher (1897) on salmon sperm and Zittle and O'Dell (1941) on bull sperm. The protein material appears to be largely in the " alpha " form, and hydroxyproline is absent, so that the protein could not be collagenous (Tibbs). In addition to the protein material, both cilia and flagella, though not sperm tails, contained a small proportion of nucleotide material, mainly as ribonucleoprotein, but, while Child found about 2·5 per cent of RNA, Watson *et al.* only found about 0·3 per cent in the same material. Enzymatic activity in the form of an apyrase capable of splitting phosphate from adenosine phosphates was found by Child and Tibbs, although Child found that the cilia preparation was most active on adenosine-5-monophosphate, while Tibbs' flagellar extract was more active on adenosine triphosphate and adenosine diphosphate and much less active on adenosine monophosphate and beta-glycerophosphate (see also p. 108). A small amount of carbohydrate material was present in both cilia and flagella.

Earlier workers had found that the ciliary or flagellar fibrils were digested by proteolytic enzymes, e.g. Pitelka and Schooley (1955) found that the axoneme (fibre bundle) of *Euglena* was disrupted by trypsin. Grigg and Hodge (1949) even found a difference between the central and peripheral fibrils, for the two central fibrils of the cock sperm were more easily destroyed by pepsin than the peripheral fibrils. The central fibrils of sperm of *Psammechinus* are also more easily disrupted than the peripherals (Bradfield, 1955) and so may be of a different constitution.

The very large comb-plates of ctenophores, with cilia up to 2 mm long in clumps of 100,000 or more cilia should offer ideal material for some biochemical and biophysical studies.

Bacterial flagella, which are also proteinaceous and have been studied by several groups of workers (e.g. Astbury and Weibull, 1949; De Robertis and Franchi, 1951), are probably not identical in composition to true cilia and flagella, for Tibbs (1957) found that *Polytoma* flagellar protein contained the amino acids cystine and tryptophan which are believed to be absent from bacterial flagellar protein (see also p. 76). Neither of these amino acids was found in *Tetrahymena* cilia by Watson *et al.*, perhaps because their hydrolytic technique would not permit the recognition of these acids, but both were found in *Chlamydomonas* by Jones and Lewin (1960); evidently the amino acid composition of cilia and bacterial flagella requires further study.

Root structures associated with cilia are doubtless also proteinaceous in nature (Grimstone, 1961), although little work has been done to confirm this.

3. Variations in Shaft Structure

Cilia

Some cilia have been markedly specialized, especially where they have a non-motile function. The shafts of normal motile cilia are found to vary little from the pattern described above for a typical cilium. Cilia are usually grouped to perform their special functions and their co-ordinated action produces the characteristic water movements. Usually many single separate cilia work together with metachronal co-ordination, but, when such single cilia cannot produce a large enough force, compound structures may be built up of many cilia which can function as a single unit; these compound cilia may still be metachronally co-ordinated with other compound cilia, or each compound cilium may beat independently.

The details of structure of several types of **compound cilia** that have been studied under the electron microscope show features not found in typical cilia. In the ciliated protozoa, compound cilia may be either cirri, which have the shape of a truncated cone and occur in fairly small numbers usually without metachronal co-ordination, or membranelles, shaped like triangular plates, which occur in rows in which their beat is metachronally co-ordinated. The shafts of the cilia in the ciliate type of undulating membrane (not to be confused with the completely different undulating membrane of some flagellates and sperm tails, (see pp. 38 and 42) occur close together in a long single row, but it is not clear whether they are joined in any way (King, Beams, Tahmisian and Devine, 1961).

Cirri of *Euplotes* are built up of 20 to 35 cilia hexagonally packed in 5 to 8 rows (Pl. Va), but Chen (1944) found as few as 4 cilia in some cirri of *Stylonychia*. The membranelles of *Stentor* and *Euplotes*, shown in Fig. 8 and Plate IVa respectively, are built up of 2 or 3 rows of 15 to 25 cilia closely packed together, while the membranelles of *Epidinium* may be larger, and contain up to 115 cilia in about 6 rows (Bretschneider, 1960). There is neither an interciliary matrix, nor any extraciliary membrane surrounding the shafts of the component cilia and holding them together to keep the shape of the compound structure. Instead, the membranes of component cilia of both cirri and membranelles carry finger-like projections up to 1 μ long and about 500 Å in diameter which ramify among the cilia of the compound structure. These projections are thought to help to maintain the integrity of the structure, but could not maintain an absolutely rigid shape. Compound cilia are also of wide occurence in metazoa, but very few have been studied, e.g. the laterofrontal cilia of *Mytilus* which are rather like membranelles with two fairly close rows of about 20 cilia (Bradfield, 1955). The laterofrontal cilia of *Anodonta* figured in Plate IId are very similar, and all the cilia are seen packed tightly together.

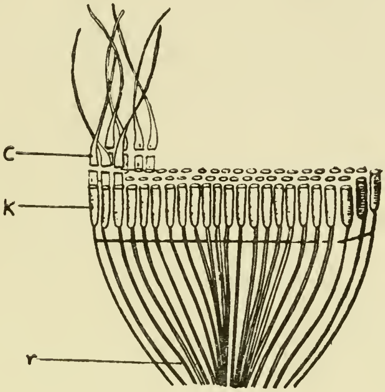


FIG. 8. Diagram of the arrangement of kinetosomes in a membranelle of *Stentor*. Each kinetosome (*k*) bears a ciliary shaft, (*c*) at its distal end and a number of root fibrils (*r*) at its proximal end. The cilia are not drawn to scale (from Randall and Jackson, 1958).

Perhaps the most remarkable compound cilia that have been described are the comb-plates of the ctenophore *Mnemiopsis* studied by Afzelius (1961a). Each comb-plate of *Mnemiopsis* functions as a single unit and contains several hundred thousand cilia hexagonally packed in 80 to 100 rows, each of several thousand cilia. Again there is no evidence of any membrane or sheath surrounding the compound structure, nor are there any projections of the ciliary membrane, but the cilia show an unusual internal structure which may be important in uniting cilia in rows parallel to the longer sides of the comb-plates. The cilia within each row are very close together (with a separation of as little as 30 Å), and two peripheral fibrils of each cilium (numbers 3 and 8) are connected to the ciliary membrane by a longitudinal lamella about 80 Å thick and 500 Å long (Pl. Vb). These compartmenting lamellae, as Afzelius has called them, terminate on their respective ciliary membranes exactly opposite the lamellae of adjacent cilia in the row, and in some cases the narrow interciliary space between the lamellar endings seems to contain a dense material that might cement the ciliary shafts together along these longitudinal lines. There seems to be adequate evidence that these lamellae have a function in holding the cilia together, for many of the cilia at the ends of the rows have lamellae on their inner side where there is a neighbouring cilium, but not on the free outer side. Also, where cilia of a row are pulled out of line, the lamellae may be stretched and distorted so that they still end opposite each other. Normally the compartmenting lamellae of all the cilia of any row are more or less in one line; this line also passes through both central fibrils of each cilium, and is at right angles to the plane of beat of the comb-plate. Ctenophores also bear cilia on other parts of the body which are not compound and do not have the lamellae.

The cilia of ctenophore comb-plates show two further peculiarities. At the centre of the cilia is a mid-filament (see p. 17 and Fig. 5b), which appears in some transverse sections as an approximately rectangular block about 180 Å by 100 Å. It occupies the same position in relation to the central fibrils in all cilia of a comb-plate. Cilia at the edge of the two long sides of the comb-plates often contain a number of intraciliary vesicles with membranes about 65 Å thick (the same as the ciliary membrane) and sometimes with several concentric membranes.

Sensory cilia often lack the central fibrils. The rods and cones of the vertebrate eye are composed of two segments connected by a narrow neck about 0·5 to 1·5 μ long and about 0·2 μ in diameter (Pl. VIa, b and c) (Sjöstrand, 1953; De Robertis, 1956a; De Robertis and Lasansky, 1958; Tokuyasu and Yamada, 1959; Eakin and Westfall, 1959, 1960). This neck contains a ring of nine longitudinal fibril doublets which have their origin at a typical basal body in the inner segment and terminate in the outer segment, where they may make contact with the edges of the flattened sacs which form the photoreceptive part of both rods and cones. There is no well-defined fibrillar material at the centre of this fibril bundle. The " connecting cilium " forms the only contact between the receptive part of the cell and the dendritic nerve ending, so that some part of the cilium must conduct the impulse generated in the outer segment. Striated roots from the basal body run deep into the inner segment.

Fauré-Fremiet (1958) has pointed out the parallel structural developments found in the rods and cones of the vertebrate eye and the photoreceptive region of the phytoflagellate *Chromulina psammobia*. A short internal flagellum runs from the base of the emergent flagellum to lie alongside the eye-spot chambers; this may conduct information from the eye-spot to the locomotor flagellum.

The distal sense cells of the eye of *Pecten* carry curious oval appendages about 1μ in diameter. Transverse sections show concentric banding of alternate light and dark zones which diverge from the appendage at the outside and end in the cytoplasm. These terminations have the structure characteristic of ciliary basal bodies; and from them ciliary shafts with nine dense loci run to the appendage, apparently forming its whole struture. The presence or absence of central fibrils in the cilia does not seem to have been established for certain by Miller (1958) who has described this structure. It is suggested that these appendages form the receptive part of the sense cell.

Until recently it was believed that cilia were not present in arthropods, then insect sperm (Bradfield, 1955), the scolopale organ of the locust (Gray and Pumphrey, 1958; Gray, 1960), and the plate organs on the antenna of the honey bee (Slifer and Sekhon, 1960), were found to contain the typical ciliary structure. The scolopale sense organ detects vibrations of the tympanic membrane of the insect " ear". This is not an appropriate place to describe the detailed struture, but only the occurrence and structure of the cilium. Sensory dendrites of the scolopale organ end in cilia about 12 μ long which run in an intercellular space for most of their length (Fig. 9), and then end in a deep pit in a cap

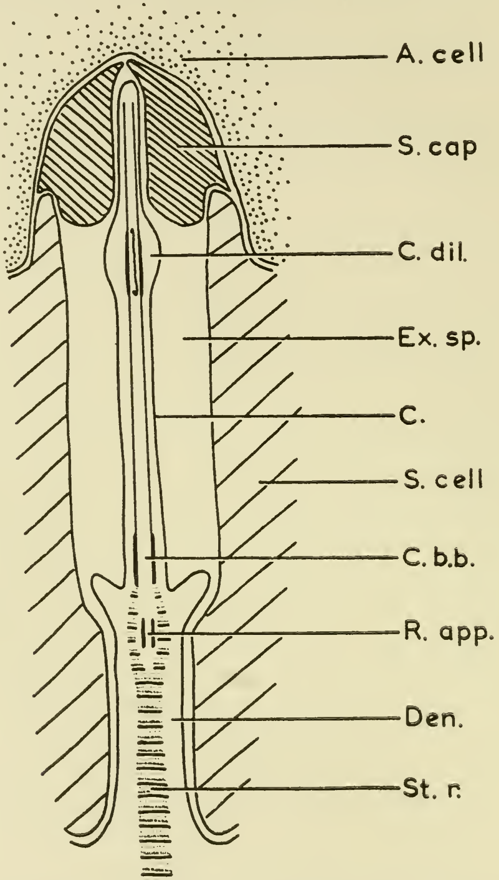


FIG. 9. Diagram showing the relationship between the cilium and the dendrite, scolopale cell and scolopale cap in the auditory ganglion of the locust.

A. cell, attachment cell; *S. cap*, scolopale cap; *C., dil.*, ciliary dilation; *Ex. sp.*, extracellular space; *C.*, cilium; *S. cell*, scolopale cell; *C.b.b.*, ciliary basal body; *R. app.*, root apparatus; *Den.*, dendrite; *St. r.*, striated root (after Gray, 1960)

structure which is more or less rigidly connected to the tympanic membrane. The cilium contains a ring of nine fibril doublets, but the centre of the axial bundle is empty except for an irregular fibrous structure in a dilated region towards the distal end. From the basal body of the cilium nine striated roots run downwards for a short distance (Pl. VId) before joining to form a thick single root which runs deep (80 to 100 μ) into the dendrite with repeated branching. Here again the cilium has a conducting function, and indeed seems to generate the receptor impulse.

Other cilia which could have a sensory function are those on the crown cells in the saccus vasculosus of fish. Here again the central fibrils are missing and the cilia are non-motile. The free part of the cilium is club-shaped and the peripheral fibrils which are obvious near the base extend towards the tip as strings of vesicles (Bargmann and Knoop, 1955; Porter, 1957). The function of these cilia is not obvious, but, since the saccus vasculosus is believed to act as a pressure receptor, the bulbous cilia may be the receptor organs.

The cnidocils of nematocyst cells of *Hydra* have been shown by Chapman and Tilney (1959) to have a central dense core with nine peripheral ridges which may represent the peripheral fibrils of a modified cilium. At the base of this structure is a typical basal body.

Wersäll (1956) found normal cilia as well as "stereocilia" (see p. 74) on the hair cells of the cristae ampullares in the guinea pig ear. The normal cilia contain the full complement of nine-plus-two fibrils and run for most of their length embedded in the gelatinous material of the cupola. It is thought that movements of the cupola, caused by movements of fluid in the ampullar cavity, distort the cilia and cause electrical discharge in the sensory nerve. Cilia with the full number of fibrils are also found on the olfactory rods—the receptive endings of the olfactory nerves—of the rabbit (de Lorenzo, 1957).

An important skeletal function is performed by certain **supporting cilia** in protozoa. Ciliary rootlet structures are sometimes enlarged to form skeletal structures in protozoan cells, e.g. the kinetodesmata of *Metaradiophrya* (p. 63), the costa of *Tritrichomonas* (p. 71), and occasionally the cilia themselves may form a main part of a skeletal structure. The stalks of several peritrich ciliates have been found to have ciliary origin by Rouiller, Fauré-Fremiet and Gauchery (1956). The stalks of the non-contractile types *Campanella* and *Opercularia* and the contractile *Zoothamnium* are long cylindrical structures, with a central contractile " spasmoneme " in the third-named genus. An aboral scopula of cilia forms a fibrous skeleton in the stalk, while other parts of the peritrich surface produce matrix material to fill the interstices. The detailed structure is different in all three genera.

The cilia which elongate into the stalk of *Campanella* have a normal fibrillar structure near the base, but soon widen out and lose all the inner fibrils. The cilium continues down the stalk as a hollow cylinder formed from the ciliary membrane which is interconnected with other similar cylinders by oblique trellis-like fibrils about 50 to 100 Å thick. In the other two genera the cilia again elongate into the stalk, but the peripheral fibrils form the skeletal structure, the ciliary membranes disappearing. In *Opercularia* the nine peripheral fibrils diverge somewhat near the base and become striated, the striations having a period of about 440 Å in the fully developed stalk. The nine peripherals of *Zoothamnium* again diverge, but then fuse to form a single cylinder about 3500 Å in diameter with a striated wall having a 470 Å period. Although central fibrils are absent in *Opercularia*, a non-striated structure which may represent the central fibrils has been seen in *Zoothamnium*.

These details of structure found by Rouiller, Fauré-Fremiet and Gauchery form a coherent picture of stalk structure in these three peritrichs, but Randall (1962) has studied a wide variety of peritrich stalks and believes the picture described above to be an over-simplification. He has obtained striking electron micrographs which show that the striated fibres are not continuous with the peripheral fibrils of the scopula basal bodies. Further work is obviously necessary before we can fully understand these structures.

Flagella

A variety of modifications of the flagellar shaft have been described; most of them have the result of increasing the effective diameter of the flagellum. Hairs or scales may be present on the flagellar surface, the membrane may be expanded, or additional material may be longitudinally disposed either inside or outside the flagellar membrane. Internal modifications in the form of spines on the fibrils and connexions between the fibrils and the flagellar membrane have also been found.

Many light microscopists described "Flimmergiessel" or **flimmer-flagella** (Brown, 1945), which are flagella bearing hair-like appendages. Flagella were classified by Deflandre (1934) and Vlk (1938) on the basis of shape and the distribution of hairs. Work with the electron microscope has shown that these appendages fall into two distinct groups, the thicker "mastigonemes," which are from about 1 μ to a few micra long and 100 to 200 Å in diameter, and much finer filaments which form a felted zone around the flagellar shaft in some species. Information is very scanty on these fine filaments which have been figured by Pitelka and Schooley (1955), and appear as a zone about $0 \cdot 2\ \mu$ thick around the anterior flagellum of *Peranema* in electron micrographs taken by Roth (1959) (Pl. VIIb). Fine filaments of this type have been reported from the flagella of the sponge *Microciona* by Afzelius (1961b,c), who found filaments about 20 Å thick and $0 \cdot 2$ to $0 \cdot 3\ \mu$ long arranged in two rows in the plane of the central fibrils as shown in Plate VIIc.

Mastigonemes occur in a single row on the anterior flagellum of euglenid flagellates, and perhaps on the transverse flagellum of dinoflagellates. In the most frequent arrangement there are two rows along opposite sides of the flagellum as on the anterior flagella of spermatozoids and zoospores of brown algae, yellow-green algae, and some fungi, and on flagella of a variety of flagellates (Pl. VIII, IX) (Manton, 1956; Pitelka and Schooley, 1955). The extent of mastigonemes along the flagellum may vary; a greater or lesser region at the tip of the flagellum may be devoid of flimmer hairs. The flagella of the animal-like flagellates lack mastigonemes as do also the posterior flagella of most biflagellate forms, though these may bear some of the fine filaments. Most mastigonemes have two regions, a stouter basal region ending in one or more fine terminal filaments (Pl. VIIIa), which are usually short but may be as long as the basal part. There is doubt as to the position of attachment of the mastigonemes; Manton (1956) is of the opinion that they are attached to the peripheral fibrils of the shaft, or between a pair of such fibrils, while Pitelka and Schooley (1955) find that in some cases at least the mastigonemes seem to arise from the sheath. Certainly, in some of Manton's micrographs of frayed cilia, the mastigonemes seem to be consistently associated with only one or two of the peripheral fibrils as if they were attached.

The **scales** on the flagella of the algal flagellate *Micromonas squamata* described by Manton and Parke (1960) are a more unusual feature (Pl. VIIIb). These scales are circular with a diameter of 0·15 to 0·4 μ, and are very thin with thickenings in the form of a spider's web pattern. They cover the flagellum in a regular imbricated pattern and also occur on the body, but their means of attachment is uncertain. There is some evidence that they are formed inside the body in vesicles and extruded from the surface.

An expanded flagellar membrane may be flattened to form a fin, a ribbon-shaped flagellum or an undulating membrane. The posterior flagellum of the second stage zoospore of the fungus *Saprolegnia* has a large membrane which, at least after drying, has the appearance of a lateral fin, and, after the removal of the membrane, some flagella show a row of fine hairs about 0·5 μ long that could support the membrane of the fin (Manton, Clark and Greenwood, 1951). The transverse flagellum of the dinoflagellate *Gyrodinium* is ribbon-like with a flagellar membrane that is expanded in one plane to make a flat band 2 to 3 times the width of the internal fibril bundle (Pitelka and Schooley, 1955). Confirmation by means of sections is obviously necessary for both of these structures.

A number of animal-like flagellates have an **undulating membrane** in which the motile organ seems to be a single flagellar shaft towards the outer margin. In the undulating membrane of *Trichomonas* (Pl. VIId), the greatly expanded flagellar membrane contains some material with a fibrous appearance in addition to the flagellar fibre bundle, and is united with a thin fold of the cell membrane for much of its width (Grimstone, 1961). The fold of the cell membrane has not been found by either Anderson and Beams (1959) in *Tritrichomonas* (=*Trichomonas*), or Vickerman (1962) in trypanosomes; in both cases the axial bundle and additional fibrous material were found inside an expanded flagellar membrane which may be closely applied (and adherent?) to the body surface. Vickerman found that the additional intraflagellar material of *Blastocrithidia* was striated or perhaps hexagonally cross-linked (Pl. VIIf).

The anterior flagella of the spermatozoids of some of the brown algae bear stout **spines** on one of the peripheral fibrils. In *Himanthalia* there is a single large spine 1 μ long near the distal end of the flagellum (Pl. IXb,c), while the flagellum of *Dictyota* bears about 12 smaller spines spaced at more or less equal intervals along one fibril (Manton, Clark and Greenwood, 1953). Manton (1956, 1959c) believes that the spines are borne on fibril number 1, the fibril that lies in the plane of bilateral symmetry. The function of these spines is uncertain, though they may be used in fertilization as Manton suggests.

Additional internal material is present between the fibril bundle and the membrane in shafts of a number of flagella. A thick tapered strand of **intraflagellar material,** up to 0·5 μ in diameter near the base, runs alongside the fibril bundle in the flagella of *Euglena* and *Peranema* (Pl. VIIa,b) (Roth, 1958a, 1959). No definite information is available about the structure of this material, though in some cases it is fibrillar and Roth found it to originate from a kinetosome-like body, so that it may be comparable with the material of the fin of amphibian sperm (p. 42). Incidentally, the basal 1 μ of the flagella of *Euglena* and *Peranema* were found by the same author to lack the central fibrils of the fibril bundle, which is in this region swollen to about twice its normal diameter (at least in fixed preparations). The central fibrils are also lacking in the basal region of trypanosome flagella, where there may be a complex transition region (Pl. XIIc and d).

Intraflagellar material in *Pyrsonympha*, where the 4 to 8 flagella run in spiral grooves along the surface of the animal, takes the form of peripheral dense strands, usually triangular in section, opposite some of the peripheral fibril doublets (Pl. VIIe) (Grassé, 1956). The flagella seem to adhere in the grooves in some way, and may have attachments similar to those of the proximal parts of flagella of *Pseudotrichonympha* to the walls of grooves on the body.

The flagellar bases of *Pseudotrichonympha* occur in rows at the bottom of longitudinal grooves, and there are connexions between the flagella and the walls of the groove, and also occasionally between adjacent flagella (Gibbons and Grimstone, 1960). These connexions are reminiscent of the compartmenting lamellae in the cilia of ctenophore comb-plates (p. 32). Granules about 180 Å in diameter are found on the inside of the cell membrane of the wall of the groove, exactly opposite some of the peripheral fibrils of the flagella (Pl. I). Fine filaments run across the extracellular gap from these granules to a small dense mass inside the flagellar membrane, and probably penetrate both membranes. From here they can be traced to a termination at a ridge of dense material at the junction between the two subfibrils on the outer side of a peripheral fibril. Gibbons and Grimstone interpret these linkages as filaments rather than lamellae because distinctly separate granules can be seen on the groove wall in longitudinal sections. The rare interflagellar connexions of *Pseudotrichonympha* link peripheral fibril 3 of one flagellum to peripheral fibril 8 of the next flagellum (cf. ctenophore comb-plate) by means of a filament that crosses an interflagellar gap of 100 Å or more.

Manton (1959a) has described the unusual shape of the flagellum of the minute flagellate *Chromulina pusilla*, which deserves a mention, although it appears to be based on a normal constructional plan. The whole flagellum is only about 3 to 4 μ long and is sharply divided into two parts, with a 1 μ long basal portion about 1500 Å in diameter containing the normal 9-plus-2 fibril pattern connected to a normal basal body, while the distal 2 or 3 μ form a long whiplash about 500 Å in diameter which apparently contains only the two central fibrils (Pl. VIIIc).

Outside the flagellar membrane of the anterior flagellum of *Peranema* are two longitudinal half-cylindrical **sheaths** of striated material. These have been described by Chen (1950), Pitelka and Schooley (1955) and Roth (1959). The electron micrographs published by Roth show that in transverse sections the sheath appears to be built up from 4 to 6 layers about 100 Å apart and 100 Å thick, composed of material that in longitudinal section has a transverse striation with a periodic spacing of about 450 Å (Pl. VIIb). This structure deserves further study at high magnification to discover its means of attachment to the flagellum and perhaps finer details of structure.

Sperm Tails

Some animal sperm lack flagellar tails, e.g. the sperm of the crustacean *Procambarus*, in which, nevertheless, normal centrioles are present (Moses, 1961). The basic pattern of the 9-plus-2 axial fibril bundle has been found in the tails of most sperm examined, but is nearly always supplemented in some way for at least part of the length of the tail. Mitochondria are very often found in the tail, either as a sheath surrounding it for a greater or lesser part of its length, or as strands running along the tail. These mitochondria are usually modified during development and lose the usual arrangement of internal cristae, e.g. in the rat sperm (Yasuzumi, 1956); this may allow the storage of a large quantity of substances with readily available energy. Similar modifications occur in the fern *Pteridium*, where the mitochondria are retained within the body of the multiflagellate spermatozoid (Manton, 1959b). Another sheath of a fibrous or granular material is found surrounding the tail for much of its length, especially in mammals, where an additional set of nine fibres outside the peripherals is also found in the proximal part of the tail. The sperm tail of the ostracod does not follow the usual plan (Bradfield, 1955), and deserves further study.

Afzelius (1959) found that the tail of a sea urchin sperm is structurally an unmodified cilium. At the base is a cylinder of fibril triplets, and the shaft has two central fibrils with radiating strands to the nine peripheral doublets, each of which carries transverse arms on one subfibril; the whole shaft is surrounded by a three-layered membrane (Pl. Xa). Around the base of the tail is a mitochondrial sheath about 0·5 μ long and 1 μ in diameter, which fits against the nucleus to form the posterior part of the sperm head (Rothschild, 1956). The sperm of the annelid *Hydroides* is very similar in the structure of the tail, with two centrioles and a cluster of mitochondria (Colwin and Colwin, 1961).

Other invertebrates have sperm tails which may follow a similar simple plan with various patterns of distribution of mitochondria. Helical mitochondrial strands are present around the fibril bundle in slugs and snails, while the sperm tails of the cockroach *Periplaneta* and the bee *Apis* carry a pair of straight longitudinal strands of mitochondria (Bradfield, 1955). The fibril bundle of the sperm tail of the bee, which appears to have enlarged and modified peripheral fibrils, is also accompanied by a pair of additional strands of uncertain structure inside the tail membrane (Pl. XIb) (Rothschild, 1955). An additional ring of nine outer dense fibrils is present immediately outside the peripheral doublets in the mid-piece of sperm of the snail *Helix* (Grassé, Carasso and Favard, 1956). This mid-piece carries a sheath of several concentric layers of membranes inside the mitochondrial helix, and a sheath of longitudinal fibrils (200 Å in diameter) around the outside.

A modification of the central part of the axial fibril bundle has been found in the sperm tail of the flatworm *Haematoloechus* by Shapiro, Hershenov and Tulloch (1961). Here the usual pair of fibrils is replaced by a single structure made up of three parts, which have an overall diameter of 500 Å. At the centre is a dense core surrounded by a less dense cortical region, around which is a sheath with some indications of a fibrillar structure. The authors believe that there is a connexion between spiral striations of this sheath and the prominent radial spokes running from the central structure to the nine normal peripheral doublets (Pl. Xb, c). The spokes are found to occur at intervals of about 220 Å along the axis. Another interesting feature of this sperm tail is the sheath which is said to be made up of 36 longitudinal fibrils (about 200 Å in diameter), but these were not present in all of the micrographs presented and may enclose only a specialized part of the tail as in *Helix*.

Most of the **vertebrate sperm** studied have been obtained from mammals, but some important variants of structure have been reported from other vertebrates, particularly from the amphibians, while fish sperm (Lowmann, 1953) and bird sperm (Grigg and Hodge, 1949) seem to follow the normal pattern. The sperm tails of the toad *Bufo* (Burgos and Fawcett, 1956) and the newt *Triturus* (Fawcett, 1958b; 1961) both show a large lateral fin running the length of the tail in the plane which passes through the two central fibrils of the axial bundle. In *Bufo* this fin is composed of a thin layer of fibrous material which extends from a thickened strand that runs alongside the axial fibril bundle to another thick strand that runs along the outer edge of the fin, the whole being surrounded by the tail membrane (Pl. XIa). The maximum width of the fin is about 1 μ and it narrows towards the base of the tail, where the fibrous material is said to have its origin at a second centriole (cf. the intraflagellar material in

Peranema described on p. 39). Burgos and Fawcett observed that the fin of *Bufo* is capable of the propagation of waves of movement that can be independent of those of the main axis.

The fin of *Triturus* differs in that the main axis of the tail is formed by a very thick strand of fibrous material which may be horseshoe-shaped or trifoliate in section, and is accompanied for part of its length by mitochondria. The axial fibre bundle runs along the margin of the unthickened fin; movement of this flagellum can cause undulations of the fin like those of the undulating membrane of *Trichomonas*, while the main axis of the tail remains stiff (Fawcett, 1961).

Sperm tails of mammals, both eutherian and marsupial, can be divided into four regions: neck, mid-piece or middle-piece, principal-piece and end-piece. The neck includes the basal centrioles and forms a connexion between the head with its nucleus and the mid-piece which is characterized by the presence of a mitochondrial sheath. (The name mid-piece may also be used in non-mammalian sperm for regions bearing a mitochondrial sheath.) The principal-piece is surrounded by a more or less spiral sheath of dense material, which may be fibrous or granular, while the end-piece has no sheath, the fibril bundle being surrounded only by the membrane which covers the whole of the sperm. The relative lengths of the various regions differ in different species, e.g. there is no end-piece in the bandicoot sperm (Cleland and Rothschild, 1959), but the character of each region is fairly constant. The excellent account of the structure of the human sperm by Ånberg (1957) will be used as an example for a description of the various regions; a set of diagrams by the same author is reproduced in Fig. 10.

The axial fibril bundle that runs the length of the tail has the normal arrangement of tubular fibrils. Radial strands run from the two centrals, which lie in a slightly denser region of the matrix, to the nine peripherals, of which the two subfibrils can be seen to be arranged circumferentially and not radially as Bradfield (1955) suggested. Towards the tip of the end-piece the fibrils become single and are irregularly scattered in the matrix.

Outside the peripheral doublets, and on the same radii, is an additional ring of nine coarse fibres of a homogeneous dense composition, which extend from the neck through the mid-piece

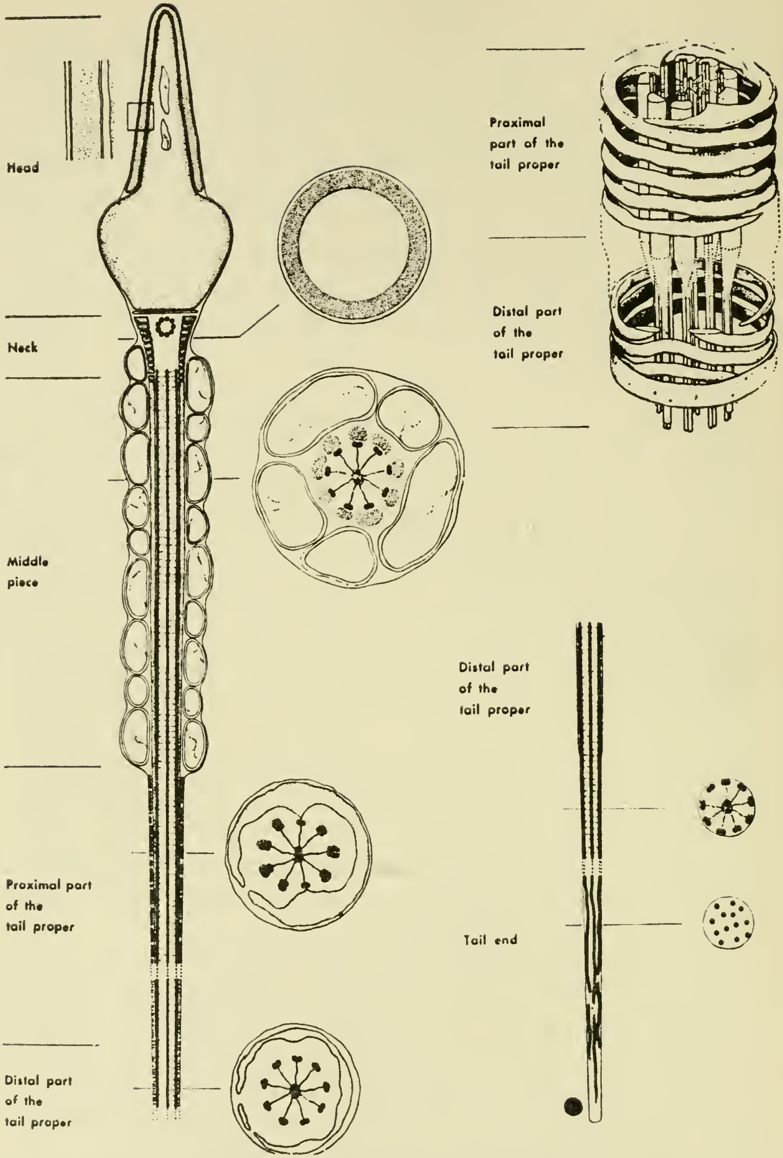

Head
Neck
Middle piece
Proximal part of the tail proper
Distal part of the tail proper
Proximal part of the tail proper
Distal part of the tail proper
Distal part of the tail proper
Tail end

FIG. 10. Tentative diagrammatic reconstructions of the structure of the human sperm, showing structures in longitudinal and transverse sections, and a three-dimensional view of the sheath and fibrils of the principal-piece (from Ånberg, 1957).

to end part of the way along the principal-piece. These nine outer fibres are all of similar size in human sperm, though not in some other animals (see below), and are thought to be built up of many filaments about 75 Å thick. In the neck region the anterior extremities of the coarse fibres are segmented into a series of transverse bands, in each of which all nine fibre blocks have been fused to form a ring. This curious segmented structure surrounds the base of the axial bundle and the centrioles. The two outer fibres nearest to the plane in which the centrals lie are the shortest, and end before the others posteriorly. There is uncertainty about the origin of these outer fibres, though similar structures are found in the flagella of *Pyrsonympha* (p. 39) as well as in invertebrate sperm (p. 41).

Both of the tail sheaths of the human sperm may appear spiral, but in neither case is the structure a simple helix. The modified mitochondria of the fully developed sperm are moulded to fit closely around the fibril axis, and may be arranged in a spiral manner without the coalescence into a spiral that has frequently been described. The sheath which surrounds the principal-piece is built up of a thick spiral fibre which frequently splits, diverges and rejoins. Each gyre of this fibre is markedly thickened in the plane of the two central fibrils, so that the sheath has an oval rather than a circular outline. Fawcett (1958a) and Cleland and Rothschild (1959) have found that in various mammalian sperm tails the successive gyres of the spiral are linked longitudinally along the two lines of thickenings, but Ånberg did not find this. In fact, Fawcett believed that the sheath was formed from two rows of semicircular ribs running between two longitudinal strands and surrounding the fibril bundle. For much of the length of the principal-piece, the spiral sheath is in contact with the axial fibrils by means of projections from the thickened regions which extend towards the nearest peripheral fibrils, and are therefore near to the plane through the central fibrils. The spiral fibre decreases in thickness posteriorly and there lacks connexion with the axial fibrils.

Cleland and Rothschild (1959) have worked out the complex structure of the principal-piece of the bandicoot (*Perameles*—a marsupial) sperm tail. The " lozenge-shaped " ($1 \cdot 73 \times 1 \cdot 45\ \mu$) cross-sectional outline of the tail shows a curious asymmetry of the fibrous sheath, inside which is a normal 9-plus-2 bundle of fibrils surrounded by nine very large outer fibres (Fig. 11a). These large fibres have a homogeneous dense appearance in sections, and vary in size, shape, distance from the centre of the tail and connexions to other components of the tail, seven of them being joined to corresponding peripheral fibrils of the axial bundle by connecting laminae. Six of these laminae are double, with two longitudinal rows of nearly transverse filaments, 200 to 250 Å in diameter and about 250 Å apart, embedded in a matrix that is slightly denser than the other non-fibrous part of the axis; a seventh lamina is single with only one row of filaments. The

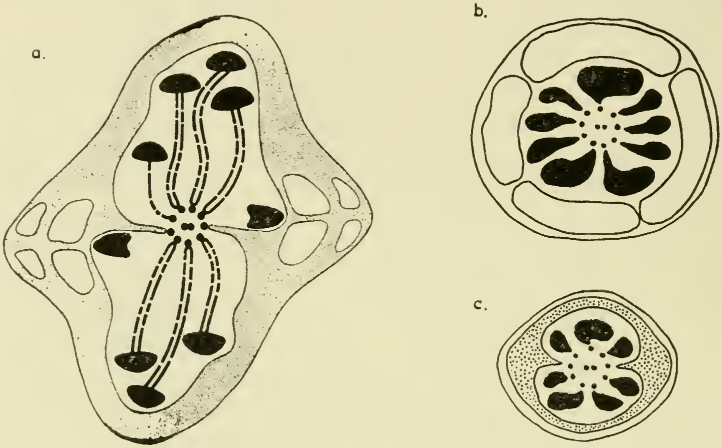


FIG. 11. Diagrams of transverse sections of some mammalian sperm tails.

a: Section through the principal-piece of the tail of the sperm of the bandicoot (*Perameles*), omitting the outer sheath structures (see text) (from Cleland and Rothschild, 1959).

b: Section through the mid-piece of the tail of the rat sperm. Note the differentiation in size of the outer fibres, the largest (1, 5 and 6) being those furthest from the plane through the central fibrils. Around these fibres is the mitochondrial sheath.

c: Section through the principal-piece of the sperm tail of the rat. There is still a marked difference in size between the outer fibres, the two in the plane of the central fibrils have disappeared and their place has been taken by laminae projecting inwards from the fibrous sheath (cf. Plate XIc).

Figs. **b** and **c** constructed from several sources referred to in the text.

remaining two large fibres, which are those nearest to the plane through the two central fibrils, lack these laminae, but they lie close to the spiral sheath where it is formed on either side into more or less continuous laminae which project inwards to make close contact with the remaining peripheral fibrils of the axial bundle.

The spiral sheath in *Perameles* is composed of two parts, the main part being a single-gyred spiral, which is tightly wound with spaces of about 300 Å between gyres. The gyres may split and rejoin, so that their width is variable (500–3500 Å); the thickness of the sheath is more constant. The second part is the pair of thickenings which occur in each gyre in the plane of the two central fibrils. Each thickening, which is roughly triangular in transverse section, contains two or three pairs of vacuoles embedded in the sheath matrix, and carries a lamina which projects inwards as mentioned above. Both parts of the sheath are made up of a dense matrix containing randomly distributed granules from 50 to 100 Å in diameter. It can be seen from longitudinal sections that there is continuity between the thickenings of successive gyres only in the region which divides the pairs of vacuoles and near the inner ends of the projecting laminae, i.e. thin connecting strands are present in the plane of the central fibrils on the minor axial plane of the tail.

Around the spiral sheath is a number of concentric layers. Two fairly dense layers 50 Å thick separated by 50 Å of less dense material occur immediately outside the spiral sheath, and are in turn surrounded by a thick (1000 Å) layer of amorphous material enclosed by the plasma membrane. Many structures are thus contributing to the massive size of this sperm tail, whose diameter is nearly 10 times that of the smallest cilium, although both have an axial bundle of fibrils of the same size and arrangement forming their central structure.

The pattern of symmetry of the axial fibril bundle found by Cleland and Rothschild in *Perameles* differs in details from that generally accepted by other authors as mentioned on p. 19. The wide separation between the axial bundle and the large outer fibres is unusual, as are the connecting laminae, although in other sperm the thick outer fibres are very close to the peripherals of the axial bundle so that connecting laminae are unnecessary. In many types of mammalian sperm (e.g. Fig. 11b) the outer fibres are seen in transverse sections to be drawn out into a daisy-petal shape, with the points touching the axial doublets, e.g. in the rat (Yasuzumi, 1956).

Differentiation in size of the outer fibres, which was also found in *Perameles*, is of more widespread occurrence. The most common pattern is that found in ram and bull sperm (Bradfield, 1955), where the three fibrils furthest from the plane through the two central fibrils (numbers 1, 5 and 6) are considerably larger than the other six. Similar differences in size are found in the rat (Yasuzumi, 1956) and guinea pig (see Pl. XIc) (Fawcett, 1958a), and Fawcett also found three slightly larger fibrils in monkey and human sperm, although Ånberg (1957) believed all nine to be of similar size. In human sperm (and also guinea pig sperm) the two outer fibres nearest to the plane through the central fibrils (3 and 8) are shorter than the other seven; this may be just as good an indication of their lesser importance as their smaller diameter might be. In rat sperm outer fibres 3 and 8 are again the shortest, fibres 4 and 7 end next, then 2 and 9, and fibres 1, 5 and 6 are the longest (Fawcett, 1961).

4. Centrioles and Ciliary Bases

The centrosome region has been known to have an important function in dividing animal cells for nearly a century, and the centriole granule in this region is regarded as the centre of production of the spindle fibres in mitotic and meiotic division of cells. The same structure is known to be important in spermatogenesis, where it is found at the base of the tail, again associated with fibrogenesis. It appears, then, as if the centriole may be functionally concerned with fibre production, at least as an organizing centre. Henneguy (1898) and von Lenhossek (1898) discussed the idea that the basal bodies of ciliary organelles were of centrosomal nature or origin. Support for this came from the finding, in several groups of flagellated protozoa, that the basal bodies of some flagella are centro-blepharoplasts which act as centrioles in mitotic division while the flagellar shafts are still attached (see the review by Bĕlař (1926) or the description of division in *Trichomonas* by Hawes (1947)). The remarkable similarity between the fairly elaborate structure of the centriole of non-ciliated cells and the basal bodies of ciliary organelles, as found in recent electron microscope studies, leaves little doubt that these two structures are very closley related. In fact, centrioles and basal bodies may be interchangeable, so that perhaps the name basal body should be replaced by centriole, as Gatenby (1961) suggested.

Although leucocytes do not show a normal mitosis, one or two **centrioles** are present in each cell, and these, as well as centrioles from other cells of vertebrates, have been studied with the electron microscope by de Harven and Bernhard (1956), Amano (1957), and Bessis *et al.* (1957, 1958). They found that the centriole is a cylinder about 0·15 μ in diameter and 0·3 to 0·5 μ long, with a less dense centre and opaque walls, in which are embedded nine groups of three longitudinal tubular fibrils (Pl. XIIa, b). Each fibril is 150 to 200 Å in diameter and, in each group the three fibrils are in a line tilted at about 40° to the tangent to the centriole exactly as in the ciliary base described on p. 24. Bessis *et al.* (1958) described additional club-shaped appendages, with a head (" massule ") about 700 Å in diameter at the end of a stalk (" pont ") 600 to 900 Å in length and 200 Å thick, which were attached to the longitudinal fibrils, about two to each fibril group. Structures of the same shape and size were found on the basal bodies of mammalian retinal rod cilia by Tokuyasu and Yamada (1959) (see Pl. VIc). Bernhard and de Harven (1960) have called these pericentriolar structures " satellites".

Frequently the centriole appears to taper slightly towards one end, which, since it is the end continuous with the flagellum in many sperm tails, is regarded as the posterior end and is equivalent to the distal end of the ciliary basal body. The centriole may be open at both ends, or closed at the posterior end by a cross-membrane, or a granule, or both membrane and granule. Peripheral fibrils of cilia and flagella are continuous with the fibrils in the centriole wall, while the central fibrils are associated with the posterior granule or membrane.

In normal somatic cells there may be a single centriole or two centrioles characteristically orientated with their axes at right angles. The tubular spindle fibres (150 to 200 Å in diameter) of mitotic cells are found to radiate from the centriole region, but their exact relationship to the centriole is uncertain. There are frequently more than 9, or even more than 27 spindle fibres, so

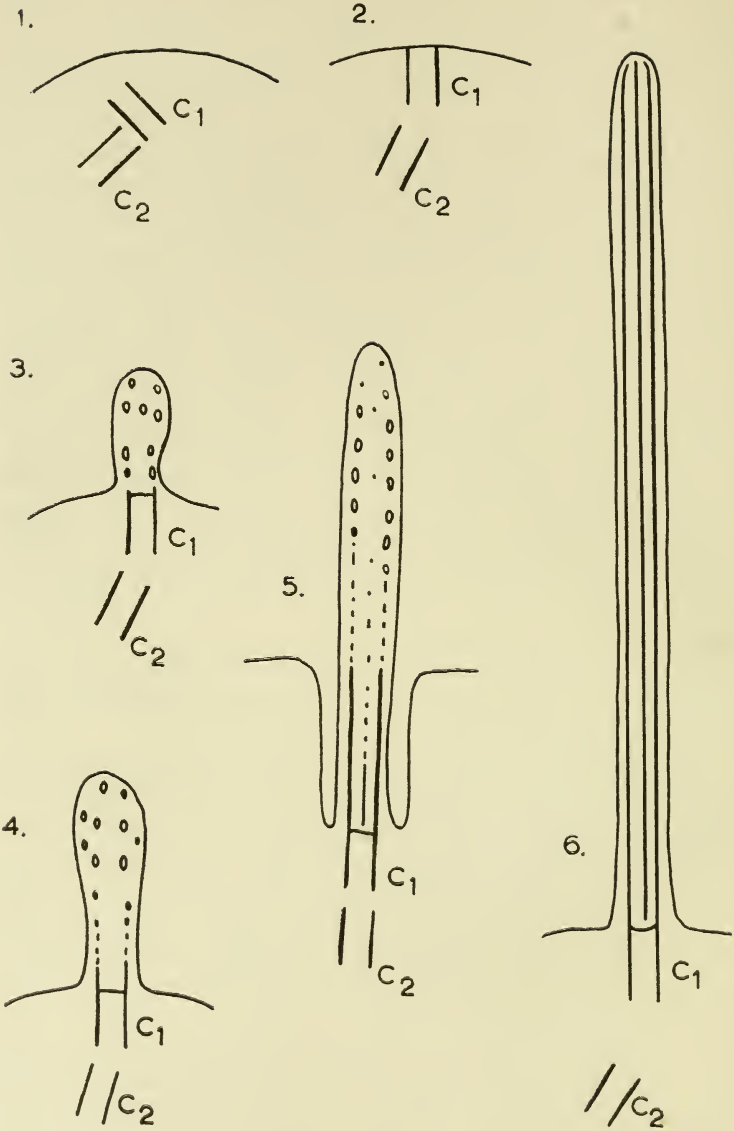


FIG. 12. Diagrams of the development of a cilium (see text). Based on the accounts of Sotelo and Trujillo-Cenóz (1958) and Tokuyasu and Yamada (1959).

that they can hardly grow out directly from the centriole wall fibrils. Amano (1957) has suggested the presence of two types of spindle fibre; nine gross spindle fibres seen under the phase contrast microscope, and fine " chromosome fibres " corresponding in number to the chromosomes present in that type of cell. According to Amano, the former type of fibril is tubular, but the latter type is not. In the meiotic spindle of the crayfish *Cambarus*, Ruthmann (1959) found that at the first division two centrioles were present at each spindle pole, and that the axes of the two centrioles and the spindle were all three mutually at right angles; only one centriole was present at each spindle pole at the second division. It is by no means certain that the spindle fibres are formed directly by the centriole, in fact Ruthmann found that the endoplasmic reticulum (as described by Palade and Porter) contributed a great deal to the spindle structure, so that the function of the centriole may be to organize the orientation of cytoplasmic components.

A rather similar appearance of the organization of cytoplasmic vesicles was found in the **developing ciliary bud** of the chick neural epithelium by Sotelo and Trujillo-Cenóz (1958) (Fig. 12). Here each epithelial cell develops one cilium shortly after its formation by the division of an undifferentiated cell. At the cell division, two centrioles orientated at right angles are associated with the nucleus of each daughter cell. After the division both centrioles migrate to the free surface of the cell, where one makes contact with the cell surface, while the other takes up a position on the same longitudinal axis as, and towards the centre of the cell from, the first centriole. The second centriole often remains transverse in sperm. Contact between the centriole and the cell membrane initiates the formation of the ciliary bud, and, for the first time in this study, a cross-membrane is seen closing the distal end of the outer centriole. There is uncertainty about the permanence of a membrane or granule in this position, for Rouiller and Fauré-Fremiet (1958) found that a granule was formed during cilium growth, while Burgos and Fawcett (1956) figure the proximal (transverse) centriole of the cat spermatid which carries a granule although it is not directly connected to the flagellar axis of the tail.

Within the fairly broad ciliary bud, which is formed by a protrusion of the cell membrane, is a cytoplasmic matrix with many small vesicles 140 Å in diameter; these vesicles may also be present inside the centriole. As the bud elongates the vesicles retreat towards the tip and the ciliary fibrils elongate from the base. It looks as if the vesicles are organized to form the tubular fibrils, but proof is not available here or in the developing connecting cilium of the mammalian retinal rod, where Tokuyasu and Yamada (1959) found strings of small vesicles in line with the developing peripheral fibrils. It is interesting that the ciliary buds of the neural epithelium closely resemble the cilia on the crown cell of the saccus vasculosus of fish, both in shape and internal organization (see p. 35) Sotelo and Trujillo-Cenóz believe that the central fibrils are formed later than the peripherals; centrals are not present in the fish crown cell cilia. At about the time that fibrils begin to appear in the cilium, the centrioles and the cilium retreat into the cell with an invagination of the cell surface. When a considerable length of the internal fibrils has been formed, the cilium emerges from the surface into the neural canal, and probably becomes functional, the basal body (centriole C_1) taking up a position just below the cell surface. This inward and outward migration of the centriole does not always occur, but traces of it have been found by Manton (1959a) in algal flagella. Also, Manton (1959c) found that before the liberation of spermatozoids of *Dictyota* from the sporangium, the single flagellum is found coiled spirally within the cell membrane, and lacks a membrane of its own; the flagellum extends from the cell before active movement starts. The actual elongation of a cilium is a fairly rapid process, e.g. 10 to 15 min. in *Ophrydium* (Rouiller and Fauré-Fremiet, 1958), and the redevelopment of a 50 μ length of the anterior flagellum of *Peranema* that had been cut off took 2 hr (Chen, 1950).

Tokuyasu and Yamada (1959) and Eakin and Westfall (1960) found two centrioles at the base of the retinal rod connecting cilium, of which one only is attached to the cilium base. The cilium of the locust scolopale organ (p. 34) has a normal basal body from which nine striated roots diverge to run around a short cylinder (Pl. VId), which has the appearance and dimensions of a second centriole, before the roots join together again to run deep into the cell (Gray, 1960). The participation of a pair of centrioles in the formation of a cilium was described many years ago by Fuchs (1904).

TABLE 4. THE DIMENSIONS OF SOME CENTRIOLES AND BASAL BODIES OF SOME CILIA AND FLAGELLA

Organism	Structure	Length (μ)	Diameter (μ)	Reference
Viviparus (spermatocyte)	Centriole	0·33	0·16	Gall (1961)
Cambarus	Centriole	0·3	0·15–0·2	Ruthmann (1959)
Melanoplus (spermatid)	Centriole	0·5	0·25	Tahmisian and Devine (1961)
Bufo (spermatid)	Centriole	0·4	0·16	Burgos and Fawcett (1956)
Vertebrate cells (various)	Centrioles	0·3–0·5	0·15	de Harven and Bernhard (1956)
Tetrahymena	Ciliary b.b.	0·3	0·2	Metz and Westfall (1954)
Ophrydium	Ciliary b.b.	0·45	0·12	Rouiller and Fauré-Fremiet (1958)
Stentor	Ciliary b.b.	0·75	0·2	Randall and Jackson (1958)
Pseudotrichonympha	Flagellar b.b.	5	0·16–0·17	Gibbons and Grimstone (1960)
Pteridium (sperm.)	Ciliary (?) b.b.	0·6	0·25	Manton (1959b)
Cycas/Zamia (sperm.)	Ciliary (?) b.b.	16–20	0·15	Barton (1962)
Anodonta	Ciliary b.b.	0·35	0·22	Gibbons (1961b)
Mus	Ciliary b.b.	0·6	0·25	Tôji (1957)
Felis (retinal rod)	Ciliary b.b.	0·2	0·12–0·17	Tokuyasu and Yamada (1959)

The centrioles that form the **basal bodies** of cilia and flagella are in general similar to those which have been described from other parts of cells, except for the structures which develop from them. Records of dimensions are available from a wide variety of animal and plant cilia, flagella and sperm (see Table 4); the diameter lies between 0·12 and 0·27 μ and the length between 0·2 and 0·8 μ, excepting in trichonymphid flagellates and cycad sperm where abnormally long structures are present (see below). The ring of nine fibril triplets in the wall of the basal body has been found in all the types of organelle, including the flagella of *Pseudotrichonympha* (Gibbons and Grimstone, 1960), the cilia of *Opalina* Noirot-Timothée, 1959) and of the rat tracheal epithelium (Rhodin and Dalhamn, 1956), the sperm of the sea urchin (Afzelius, 1959) and of the toad (Burgos and Fawcett, 1956), and will probably be found to be a general feature when improved techniques are more widely used. The transition between this triplet structure and the fibril doublets of the shaft has been described for two examples on pp. 22–26.

In the majority of cases the cavity of the basal body is cut off from the ciliary shaft by a transverse septum just below the granule at which the central fibrils end (Pl. IVb); Gatenby (1961) noted that the matrix of the shaft was denser than the matrix in the cavity of the basal body. The transverse septum may connect with the ciliary membrane, with the plasma membrane of the cell close to the base of the cilium or there may be other connexions between the axial fibre bundle and the membrane at the base of the cilium, both between the basal body and the membrane and between the peripheral fibrils of the transition region and the membrane (see pp. 23 and 25). The nine fibril groups of the basal body of the mammalian retinal rod connecting cilium are each linked to the cell membrane by a filament 600 to 1000 Å long and 100 to 150 Å thick (Tokuyasu and Yamada, 1959), while a pair of 50 Å thick filaments connect each fibril group of the basal body of *Pseudotrichonympha* flagella to the surface membrane. Filaments running between the peripheral fibrils and the membrane are also present in the proximal part of the shaft of both of these organelles.

Gibbons and Grimstone (1960) found that the basal body of *Pseudotrichonympha* flagella reaches a length of some 5 or 6 μ, of which the distal 0·6 μ differs from the proximal region in that only the latter contains a central " cartwheel " structure of filaments. The distal region may perhaps be equivalent to the whole basal body of other organelles. Fibril triplets continue through the whole structure, and, in both regions, the outer (C) subfibril of one triplet is connected to the inner (A) subfibril of the next triplet in an anticlockwise direction (looking outwards from the inner end) by a filament or lamella about 45 Å thick (Pl. I). Sometimes thinner filaments connect all the A subfibrils at the extreme distal end of the basal body. The central part of the distal region of the basal body is occupied, according to the position on the body, by regularly arranged granules or a group of three cylinders about 400 Å in diameter; the latter run from the cartwheel structure of the proximal region of the basal body to the transverse septum at the base of the shaft, and can be seen in transverse section in Plate I. At the centre of the longer proximal region of the basal body is a system of 45 Å thick filaments (or perhaps lamellae) forming a " hub " and " spokes ". The hub is a ring about 250 Å in diameter from which nine spokes run out to connect with the inner subfibril of each triplet. A similar cartwheel structure is present in the basal bodies of *Trichonympha* flagella, but is absent in the flagellar base of *Holomastigotoides*, which is otherwise similar (Pl. XIIIa).

The centriole of an early spermatocyte of the snail *Viviparus* was found by Gall (1961) to show both fibrils connecting adjacent triplets and a central cartwheel structure with hub and spokes. Gall suggested that the hub and spoke structure is characteristic of the inner or proximal end of the centriole or basal body. A cartwheel structure has been found in the basal bodies of flagella of *Blastocrithidia* (Vickerman, 1962) and cilia of *Paramecium* (Gibbons, 1960), but was not revealed in a study of *Anodonta* cilia by the latter author (Gibbons, 1961b).

The basal bodies of flagella (or cilia?) of spermatozoids of the cycads *Zamia* and *Cycas* are structures of even greater length. Barton (1962) has found basal bodies 16 to 20 μ long extending from the cell surface to a thick fibrous band underlying the spiral flagellar tract. Throughout most of the length of the basal body a spiral filament (or a succession of transverse annular filaments) was found just inside the ring of peripheral fibrils. This structure appears in transverse sections as rings rather less than 0·1 μ in diameter which are connected by filaments to each of the peripheral fibrils—like a cartwheel with a large hub and short spokes. The annular filaments were about 100 Å thick and were spaced at about 200 Å intervals along the long axis of the basal body. The peripheral fibrils appeared as doublets rather than triplets, but were tilted at an angle of about 40° to the tangent to the axial ring as in other basal bodies. At the inner end of the basal body the peripheral fibrils became joined into a fairly dense ring with nine thickenings, and the central region was occupied by a dense peg-like structure, which was about 200 Å in diameter and protruded upwards from the proximal end of the basal body for about 0·3 to 0·5 μ. Radial filaments connected the central peg with the nine thickenings of the peripheral ring. Neither the peg nor the peripheral ring seemed to be firmly connected to the fibrous band which lies underneath, although some rather amorphous material surrounded the proximal end of the basal body.

It is interesting that Manton (1959b) found a similar peg some 0·2 to 0·3 μ long and 500 Å in diameter at the inner end of the basal body of cilia of the fern *Pteridium*. The peripheral fibrils again became more or less fused at the proximal end of the basal body (which is of normal length) and were surrounded by some dense material associated with a fibrous band.

Other studies of basal body structure have not shown such detail as this. The central cavity seldom has such an ordered structure; frequently it appears quite structureless or contains amorphous masses, irregular fibres or granules. In some basal bodies the fibril triplets may lose their regular arrangement in the cylinder wall, where they seem to be embedded in a dense matrix without definite filamentous interconnexions. Studies with thinner sections and better techniques may reveal more detail here also. At the inner end of the basal body the fibrils may continue into rootlets of one sort or another, as in some mammalian cilia (Rhodin and Dalhamn, 1956); or they may end abruptly.

The arrangement of **centrioles in spermatozoa** is not entirely clear or consistent. Fawcett (1958a) mentions the presence of two centrioles in mammalian spermatozoa; these are shown in Fig. 13. A proximal centriole is situated against the posterior face of the nucleus, with its longitudinal axis transverse to the longitudinal axis of the sperm; this centriole probably has the function of forming the sperm aster in the fertilized egg. The second, or distal, centriole divides into two parts—one is a normal cylindrical group of tubular fibrils which forms the basal body of the sperm flagellum, while the second part is a short cylinder of centriole matrix material, lacking fibrils, which forms the ring centriole. After the division of the distal centriole the

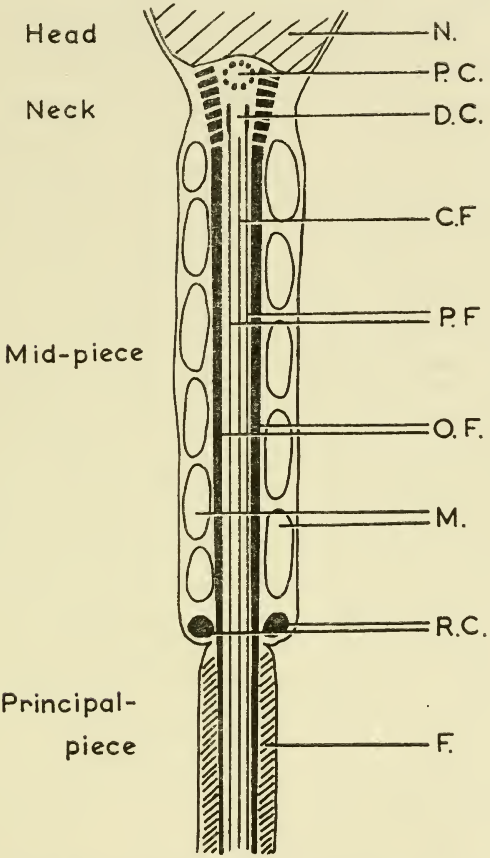


FIG. 13. Diagram showing the relationship between the centrioles, axial fibrils, mitochondrial sheath and fibrous sheath in a mammalian spermatozoon.

N., nucleus; *P.c.*, proximal centriole; *D.c.*, distal centriole; *C.f.*, central fibril; *P.f.*, peripheral fibrils; *O.f.*, outer (coarse) fibres; *M.*, mitochondria; *R.c.*, ring centriole; *F.*, fibrous sheath.

flagellum forms from the basal body, while the ring centriole, which marks the posterior end of the mid-piece of the sperm tail, moves posteriorly as a ring surrounding the axial fibre bundle, drawing out the cytoplasm to form the sheath of the mid-piece. The ring centriole, which is perhaps more correctly regarded as a centriole derivative, is seldom obvious in the adult sperm, and may only have a function in spermatogenesis. The arrangement of distal centrioles is similar in the grasshopper *Melanoplus* (Tahmisian and Devine, 1961), although in this and some other invertebrates there is no proximal centriole. Burgos and Fawcett (1956) found that in the toad *Bufo* there seems to be no ring centriole; the distal centriole forms the tail flagellum, while the proximal centriole is associated with the dense material of the tail fin. Gatenby (1961) believed that a third, unattached, centriole is also present in the amphibian sperm.

One important **function of centrioles** is the organization of fibrous structures. Thus, the basal bodies of cilia and flagella seem to be responsible for the production of the characteristic arrangement of tubular fibrils in the ciliary shaft. They also act as centres for the development of the various root systems in the cytoplasm of the cell which are described below (pp. 61–73). Rouiller and Fauré-Fremiet (1958) have found that root fibres are formed at the same time as the ciliary shafts when aboral cilia of the peritrich *Ophrydium* develop from resting kinetosomes. It is interesting that the basal body of the locust scolopale cilium produces nine normal fibril doublets outwards into the ciliary shaft, and nine striated root fibres into the dendrite cytoplasm, so that the two ends of the centriole are capable of producing different structures (Gray, 1960). On the other hand, the root structures produced by some basal bodies are tubular and not unlike the shaft fibrils in appearance and dimensions (see below). The stalks of the peritrich ciliates *Opercularia* and *Zoothamnium* (p. 36) are believed to be formed from modified ciliary shafts which develop a transverse striation, so that normal fibrils may be transformed into striated ones during development, and the basic structure of the two may not be very different.

The second important function of centrioles, that has been implicit in the theory of ciliate protozoan kinetosomes, as well as in the classical cytological picture of centrosomes, is their selfduplication. Thus Lwoff (1950) believes that one kinetosome is always formed by the division of another, without the occurrence of *de novo* formation. Grimstone (1961) pointed out that there was no direct evidence for this, only inferences from fixed material, although new basal bodies are not known to be formed in the absence of old ones, and ciliate protozoans which lose some or all of their cilia for part of their life history retain at least some of the basal bodies.

Several authors described the **division of basal bodies or centrioles,** but their evidence could not answer Grimstone's criticism satisfactorily. Gatenby (1961) wrote: " There is little doubt that the centriole divides transversely in most cases, each fibril becoming transected," and that in a micrograph taken by Tahmisian a centriole appeared to divide along its long axis. An apparent longitudinal division of the flagellar basal body has been reported by Manton (1959a) in the uniflagellate alga *Chromulina*. A third method of formation of a new basal body is by budding from the wall of the parent basal body, as suggested by some observations of Fauré-Fremiet, Rouiller and Gauchery (1956). Roth (1960) interprets his observations on division stages in *Stylonychia* as arguing against the classical kinetosome duplication theory, but this difficult study is not yet conclusive.

Studies of centrioles at various stages of development by Bernhard and de Harven (1960), and a recent study of centriole duplication in the snail *Viviparus* by Gall (1961), indicate that the budding theory may be nearest to the truth. Young snail spermatocytes carry two typical centrioles about 0·16 by 0·33 μ with normally nine triplets of fibrils in the wall. By the pachytene stage of meiosis an additional very short centriole (about 700 Å long but with more or less normal diameter) appears near one end of each mature centriole (Pl. XIIb). This procentriole, as Gall has called it, is orientated with its axis at right angles to the parent centriole, and at a distance of some 700 Å from it. It is believed to grow to full size during the late prophase; there is no direct evidence for this, although the mature centrioles are seen later to lie at right angles in a V or L formation. It is interesting that Gall has some evidence that the two ends of the centriole are functionally different, for, while the distal end forms a flagellar axis, the proximal end represents the procentriole from which the centriole grew, and is concerned with centriole replication. It is possible that centrioles or basal bodies that produce other structures from this end lose the power of replication. A hub and spokes structure has sometimes been seen at the centre of the proximal end of these *Viviparus* centrioles, and in some procentrioles.

Direct budding of the procentriole from the centriole has not been seen, and it seems possible that the development of a procentriole is " organized " or " induced " near the proximal end of the original centriole. The procentriole shows a ring of nine structures right from the start, and, if this does not originate as a thin slice from the end of an adult centriole, it is interesting to speculate on the means by which such a complex structure could develop. Bernhard and de Harven (1960) tentatively suggest that a pericentriolar satellite (p. 49) might form the daughter centriole.

A rather similar picture of the duplication of basal bodies has been revealed by Grassé (1961) in *Trypanosoma* and Vickerman (1962) in *Blastocrithidia*. Vickerman has found a short centriole lying at right angles to the basal body of the functional flagellum of a trypanosome; in other specimens two full-sized centrioles may be seen lying parallel to each other, which suggests that the short centriole elongates and turns during its development, prior to the growth of the flagellar shaft (Pl. XIIc, d). Grassé also reports the " induction " of a new centriole near the basal body of the flagellum.

Mazia, Harris and Bibring (1960) have obtained some experimental evidence that the critical time for centriole duplication in sea urchin eggs is in the early interphase or even the preceeding telophase, and we may conclude that it is at this time that the initial formation of the procentriole takes place, either by fission or by the organization of macromolecules.

Both of the centriole functions mentioned involve the production of protein structures, which, according to present theories, are only synthesized in the presence of nucleic acids. The finding of Randall and Jackson (1958) that kinetosomes of *Stentor* can be stained by the Feulgen technique, the reports that basal bodies of various cilia contain both DNA and RNA (Randall, 1959; Seaman, 1960), and many observations of granules similar to ribonucleoprotein granules in basal bodies, as reported on p. 26, could all be relevant to the synthetic ability of centrioles.

It has been reported that the kinetosomes of some ciliated protozoa may also be able to form trichocysts, which on discharge produce striated fibres with a similar appearance to some root fibres, though the periodicity of the striations is different. Ehret and Powers (1959) found that a transverse section of the tip of a young trichocyst showed an internal fibrillar arrangement similar to that in cilia, and of the same order of size, but with fewer fibrils.

5. Intracellular Structures Associated with Ciliary Bases

Almost without exception, the bases of cilia carry some sort of root structure; in some cases these were well known to light microscopists, although only electron microscope studies could show fine structural details. The bases of the flagella of animal sperm are usually closely attached to the nucleus and require no further roots, but the flagella of flagellate protozoa, algae and plant sperm are found to have roots running into the cell cytoplasm. The main function of these roots is undoubtedly one of anchorage; one has only to observe the vibration of the base of an actively beating cilium to realise that firm anchorage is essential if the cell is to remain intact. Roots usually run towards some permanent and fairly rigid cell structure, or the roots themselves may be very large, e.g. the costa of *Trichomonas* (see p. 71). Many authors have believed that some of the root structures have a conducting function; the evidence for this idea will be considered later (see p. 198).

Protozoan Cilia

Ciliated protozoa have well developed ciliary roots whose structure and function have been widely studied. All three types of root structure are found here, but in most cases they are limited to the surface layer of the body, probably because of the functions of the cytoplasm, and are modified accordingly.

A type of very short root structure is found in cirri and membranelles, where there are lateral connecting rootlets between the basal bodies of adjacent cilia. In the cirri and membranelles of *Euplotes* for example (Roth, 1956; 1957), the peripheral fibrils run the full length of the basal body (about $0 \cdot 33\ \mu$) and terminate internally at a granule 650 to 900 Å in diameter. A second, and perhaps smaller, granule is also present on the peripheral fibrils near the middle of the basal body. Solid strands run between adjacent basal bodies at their inner ends and near the middle to connect adjacent granules at the same level (Pl. IVb). A similar structure is reported for the membranelles of *Nyctotherus* (King, Beams, Tahmisian and Devine, 1961). All the component cilia of each cirrus and membranelle are thus firmly linked together at a " basal plate." The inner terminal granule of the basal body fibrils may also be used for the attachment of other types of root.

Several strata of structures of fibrillar appearance are revealed in the surface layers of many ciliates by the silver impregnation technique (e.g. Klein, 1928; von Gelei, 1932; Parducz, 1957, 1958a; see references in Corliss (1961) to many others). Ehret and Powers (1959) found that the " chicken-wire " lattice systems of *Paramecium* shown up by this technique are, in fact, parts of the pellicular corpuscles, flattened vesicular structures which are packed together to cover the surface of the animal. The fibrils connected to the basal bodies lie internal to these " silverline " lattice systems. Ehret and Powers found only the striated kinetodesmal fibres in this position, but Pitelka (1961) found also groups of unstriated fibres associated with the kinetosomes of tetrahymenid ciliates.

The cilia of *Paramecium* occur in rows or kineties, and their basal bodies are connected together within the row by the **kinetodesmata.** Chatton and Lwoff (1935) found that the kinetodesmata always run along the right (i.e. the animal's right) side of the row of kinetosomes (basal bodies), and they proposed the " rule of desmodexy," which seems to be consistent. Thus, at the surface of *Paramecium*, a kinetodesmal fibre with a striation period of 350 to 400 Å leaves each kinetosome (or the posterior member of a pair on some parts of the body surface), and runs out to the right before turning forward to join other kinetodesmal fibres in the kinetodesmal bundle. Throughout their length these fibres run more or less parallel to the body surface. Each kinetodesmal fibre extends along the kinetodesma to about the fifth or sixth cilium in front, tapering throughout most of this length from an initial diameter of 1200 Å to a point at the end (Metz, Pitelka and Westfall, 1953; Sedar and Porter, 1955). Within the bundle the kinetodesmal fibres may run straight, or may spiral around one another (Ehret and Powers, 1959).

A very similar kinety structure has been found in tetrahymenid ciliates by Pitelka (1961), although the kinetodesmal fibres may be much shorter (Fig. 14). In *Tetrahymena* for example, each kinetodesmal fibre overlaps the fibre in front by only about one-third of its length, so that transverse sections never show more than two kinetodesmal fibres in any kinety; the striated fibres again taper from about 1200 Å to a point (Metz and Westfall, 1954).

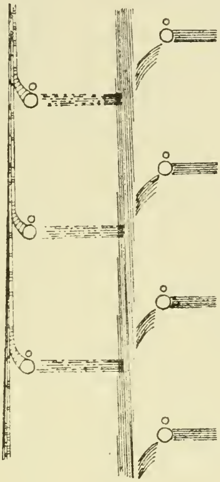


FIG. 14. Diagram of the surface view of parts of two kineties of *Colpidium campylum*. The larger circles represent the ciliary basal bodies (kinetosomes) with associated parasomal sacs (smaller circles). In the kinety on the left the striated kinetodesmal fibrils are shown, and in the kinety on the right the unstriated longitudinal, transverse and postciliary fibrils are shown; both occur in all kineties of the organism (from Pitelka, 1961).

The kinetodesmata of the astome ciliate *Metaradiophrya gigas* are composed of characteristic striated fibres, with a period of about 500 Å, which reach a much larger diameter of up to 0·5 to 0·9 μ, especially near their insertion on the attachment organ of this parasite (de Puytorac, 1959).

These kinetodesmata built up of tapered striated fibres are generally thought to be homologous with the " kinetodesmata " of such ciliates as *Stentor*, but, although they occupy a similar position, the detailed structure is certainly different. In the somatic kineties of *Stentor*, according to Randall and Jackson (1958), the non-striated fibril which leaves each basal body runs out and turns posteriorly (Pl. XIIIb) to join the " kinetodesma", which is a stack of 13 to 24 sheets of fibrils, with 24 to 30 apparently tubular fibrils about 200 Å in diameter in each sheet. Near their termination at the basal body, the fibrils bifurcate to run to either side of the basal body, and many thin filaments connect the two branches; it is thought that several of these fibrils may connect to each basal body. The " kinetodesmata " of *Spirostomum* seem to have a similar arrangement (Randall, 1957).

Unstriated fibrils with diameters between 150 and 300 Å, and with a tubular appearance in transverse section, are frequently found in protozoa, not only in association with basal bodies, but also in other structures, e.g. the pellicular ridges of *Opalina* (Noirot-Timothée, 1959), the trichites of *Prorodon* and *Coleps* (Rouiller, Fauré-Fremiet and Gauchery, 1957), the contractile vacuole canal of *Tokophrya* (Rudzinska, 1958) and in other places (see Roth, 1958a). In *Colpidium*, Pitelka (1961) has described two sets of such fibrils (about 200 Å in diameter) which arise " in intimate association with the kinetosomes." Fibrils of both groups run close enough to make contact with a group of longitudinal fibrils of similar dimensions, although connexions have not been seen (Fig. 14). The bands of short but overlapping longitudinal fibrils run parallel to the pellicle and just beneath it, superficial to and perhaps slightly to the right of the kinetodesmata. A group of 7 or 8 postciliary fibrils arises immediately posterior to each basal body, and runs posteriorly to the right and towards the surface to end near the band of longitudinal fibrils. Another group of about 6 transverse fibrils arises at the left side of each basal body and runs to the left towards the longitudinal band of fibrils of the adjacent kinety. Could it be that the postciliary and longitudinal fibrils of such a system have been elaborated to make the " kinetodesmal " structure found in *Stentor*?

The roots connected to the bases of the membranelles of *Stentor* are quite different from the somatic " kinetodesmata", although the fibrils concerned are of a similar size. Each membranelle is composed of 2 or 3 rows of 20 to 25 cilia, and the basal

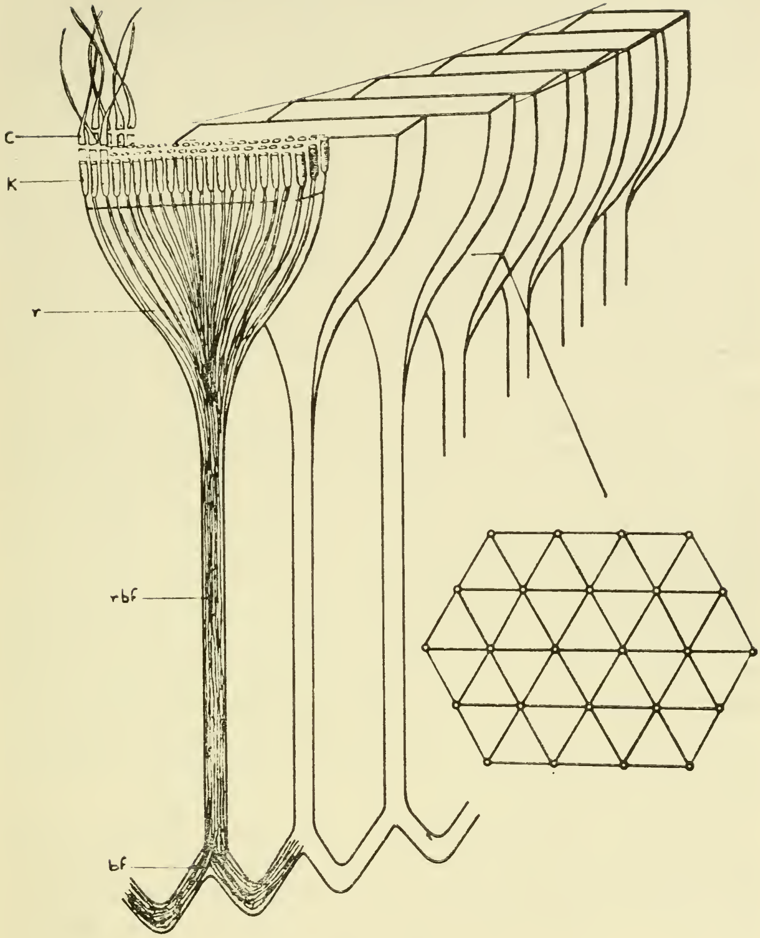


FIG. 15. Diagram of the root structures of adoral membranelles of *Stentor*. The root fibrils (*r*), which are attached to the kinetosomes (*k*) of component cilia of the membranelle, are cross-linked in hexagonal array (inset); as they run deeper into the cytoplasm, the root fibrils are formed into bundles (*rbf*) which link with the bundles from other membranelles to form the basal fibre (*bf*) (from Randall and Jackson, 1958).

body of each component cilium gives rise to about 10 fibrils which run down into the cytoplasm for about 20 μ (Fig. 15). These fibrils are regularly arranged in a hexagonal array with transverse interconnecting filaments about 60 Å thick which give a very solid structure. The fibrils from each membranelle converge into a tight bundle which bifurcates at the inner end to join similar branches from the two adjacent membranelles; the roots of all the membranelles are thus joined together at their inner ends. Another " thick strand of fibrous material " was found by Randall and Jackson (1958) joining the roots of all membranelles near the body surface, but unfortunately we do not know any details of this structure. All the information that we have about the function of these deep roots points to the fact that they serve to anchor the bases of the membranelles of *Stentor*.

A hexagonal pattern of rather similar appearance is made by rootlet connections in the infundibular region of the peritrich *Campanella* (Rouiller and Fauré-Fremiet, 1957), although in this case the structure seems to be a three-dimensional array, rather than an array of longitudinal fibrils transversely connected by filaments. The cilia are arranged in two triple rows, and the inner ends of the basal bodies give rise to groups of 2 or 3 fine filaments (10 to 20 Å in diameter) which diverge and join with those from other ciliary bases, interconnecting to form a hexagonal pattern with granular nodes at the junctions. Sections in several planes give a similar appearance. In the peritrich *Carchesium*, the bases of the cilia in each peristome row are linked together by fibrils 200 Å thick (Randall, 1956).

The roots of membranelles of *Nyctotherus* (King, Beams, Tahmisian and Devine, 1961) and the " retrociliary fibrils " which form the roots of membranelles in the Ophryoscolecidae (Noirot-Timothée, 1958), seem to be composed of bundles of fibrils similar to those forming the roots of *Stentor* membranelles, though perhaps lacking the terminal connexions between adjacent roots and the cross-filaments between adjacent fibrils. In *Euplotes*, roots of this type have not been described, but both membranelles and cirri have well-developed root systems whose rootlets run parallel to the body surface. Roth (1956, 1957, 1958a, 1958b) has described these rootlets, which are bundles of apparently tubular fibrils about 210 Å in diameter, and without cross-striations

(Pl. IV and Va), found in the positions occupied by the neuromotor fibrils investigated by Yocom (1918), Taylor (1920) and others. These fibril bundles interconnect cirri and membranelles, and Roth found a mass of intertwining fibrils at a point where fibril bundles from cirri and membranelles converge; this may be the " motorium " of some light microscopists. The pellicle covering the body surface is lined by subpellicular fibrils, again tubular and about 220 Å in diameter, running in two directions at right angles and at slightly different levels. Rootlet fibrils from the cirri also connect with these subpellicular fibrils.

Opalina is a protozoon with similarities to both ciliates and flagellates, and, although it was formerly classified with the ciliates, Grassé (1952) and Corliss (1955) have advocated that it should be grouped with the flagellates. The cilia, or flagella, of the body surface of *Opalina* are found in longitudinal rows, within which they may be connected by structures described by Pitelka (1956) and by Noirot-Timothée (1959). Some 7 or 8 short fibrils about 150 Å in diameter arise in each of two rows from each basal body and follow a slightly curved course towards the next basal body, running slightly to one side of the mid-line through the basal bodies of the row. Fibrils of one row join to form pairs with fibrils of the other row, and terminate near or possibly at the next basal body (Pl. XIIIc). If the spiral twist of the triplets of fibrils in the basal body of *Opalina* cilia shown in a micrograph (Pl. III, Fig. 2) in the paper by Noirot-Timothée follows the pattern generally found (Gibbons, 1961a), then the fibrils run forward on the left, or backward on the right of the ciliary row. Also, if Pitelka's finding that the fibrils run forward is correct, then the fibrils pass towards the left of the ciliary row and not to the right as do the kinetodesmal fibrils of ciliates. Fibrils which run backwards and to the right are known in ciliates like *Colpidium* and in the " kinetodesmata " of *Stentor*.

Metazoan Cilia

Long root structures may extend for a considerable part of the length of metazoan ciliated cells, for the roots are seldom limited to the surface layer as they are in many protozoa. The roots are nearly always of the striated type in examples so far examined, and this applies to both the specialized sensory cilia and normal motile cilia. The cilia of some epithelia of vertebrates have short non-striated rootlets.

Striated ciliary roots have been reported from most of the major groups of metazoa, viz. in vertebrates, pharyngeal cilia of the frog (Fawcett and Porter, 1954) and retinal rod cilia of the guinea pig and cat (Sjöstrand, 1953; Tokuyasu and Yamada, (1959); in molluscs, gill cilia of *Mya* and typhlosole cilia of *Elliptio* Fawcett and Porter, 1954); in ctenophores, the comb-plate cilia of *Pleurobrachia* (Bradfield, 1955); in rotifers, coronal cilia of *Philodina* (Lansing and Lamy, 1961a); in arthropods, cilia of the scolopale organ of *Locusta* (Gray, 1960); in annelids, cilia of *Lumbricus* and in ectoproct polyzoa, cilia of *Pectinatella* (Fawcett, 1958b). Generally these roots have a single origin and may branch later into many rootlets which ramify through the cell, but in the molluscs mentioned two roots arise from each basal body, and three roots leave each basal body in *Pectinatella*. The roots extend to about the level of the nucleus in most epithelial cells, while the sensory cilia in the vertebrate retinal rod and the locust scolopale organ have very long roots, extending to 80 or 100 μ in the latter case.

The appearance of the striated root of the locust scolopale cilium is shown in Plates VId and XIVb, where each repeating unit of the root can be seen to be made up of six bands. Strikingly similar bandings are seen in the roots of rotifer, annelid, (Pl. XIVa), mollusc (Pl. XIVc) and polyzoan cilia, as well as in the kinetodesmata of *Paramecium* (Sedar and Porter, 1955) and the costa of *Tritrichomonas* (see p. 71).

In *Anodonta*, Gibbons (1961b) has been able to correlate the position of attachment of structures to the basal bodies of the cilia with the direction of ciliary beat. Near the inner end of the basal body is a conical structure (the basal foot) which projects laterally from peripheral fibrils 5 and 6 (Fig. 7G and Pl. IIa, b), and is therefore on that side of the cilium towards which the effective stroke takes place. It is about $0{\cdot}15\ \mu$ long and $0{\cdot}1\ \mu$ wide and shows slight evidence of a transverse striation. In frontal cilia at least, three striated roots, one large and two small, run down into the cytoplasm from the inner end of the basal body. All three have a striation period of about 750 Å, within which up to twelve intraperiod striations may be seen in some cases, and are made up of many parallel longitudinal filaments about 45 Å thick (Pl. XIVc). The large root arises under peripheral fibrils 9, 1 and 2 (i.e. on the side away from the effective stroke of the beat), while the two smaller roots arise under fibrils 5 and 6 and shortly join the large root. At about the level at which the three roots join, the whole root structure bifurcates in the plane of the two central fibrils (i.e. at right angles to the plane of the beat) forming two long roots which run down to about the level of the cell nucleus, where they end freely. This root arrangement has interesting functional implications, for the roots are positioned in such a way that they can best resist the strains caused by the contractions of the shaft fibrils at different parts of the ciliary beat (see p. 151).

Some additional **unstriated root fibrils** are also present in the frontal cells of the gill of *Anodonta*. These fibrils appear tubular and are about 230 Å in diameter, i.e. they are like the unstriated fibrils found in ciliated protozoa, and they also resemble the central fibrils of the cilium in that they appear to consist of a two-strand helix. In some cases at least, they were observed to have connexions with the tips of the basal feet of the cilia (Pl. IIb), although the basal bodies did not appear to be regularly linked together. Gibbons could not find any similar fibrils in non-ciliated cells of the gill surface.

In epithelial cells of the mouse oviduct, Tôji (1957) found short non-striated rootlets, about 170 Å in diameter, which leave the basal body and run in a bundle into the cytoplasm. Similar rootlets found by Rhodin and Dalhamn (1956) attached to the basal bodies of rat tracheal cilia seem to be continuous with the peripheral fibrils in the wall of the basal body. In neither case were the rootlets seen to make contact with any other structure.

Flagella of Protozoa, Algae and Plant Spermatozoids

It is especially obvious in the smaller flagellated cells that the roots are serving for anchorage, since they may be attached to any of the more permanent structures in the cell, such as the nucleus, plastids and the cell surface, although this does not preclude other functions for the root fibrils. Thus, in the small unicellular alga *Micromonas*, a faintly striated fibril runs from the base of the single flagellum to end on the surface of the nucleus, while, in the similar *Pedinomonas*, four fibrils run out to the cell surface and the plastid (Manton and Parke, 1960). Striated rootlets run from each flagellar base of the biflagellate alga *Synura* to ramify over the surface of the nucleus, while, in addition, non-striated fibrils run out in the direction of the cell surface (Manton, 1955).

Rather similar rootlet arrangements are found in the zoospores and spermatozoids of the larger algae. In the green algae, the basal bodies of the quadriflagellate zoospore of *Draparnaldia* are linked by a ring of fibrils, and four rootlets bearing faint striations were found to leave the fibril ring at points equidistant from the nearest flagella and run to various parts of the cell (Manton, Clarke and Greenwood, 1955). The structure of the biflagellate spermatozoid of the brown alga *Fucus* is complicated by the presence of a peculiar probosics (Pl. IXa) whose detailed structure was described by Manton and Clarke (1956). Here the two basal bodies lie side by side at almost 180° to one another. From the region of the basal bodies, 13 fine fibrils run forward in a group away from the body of the cell, they then loop back to the surface and continue to the posterior part of the cell where they end. In the anterior loop, all the fibrils lie in the same plane to form the flat proboscis. Two shorter fibrils leave the region of the basal bodies; one runs direct to the eye-spot, while the other runs out over the surface of the proboscis.

The spermatozoid of the fern *Pteridium* is more complex, for it carries many flagella. Manton (1959b) found that the spiral backbone of the spermatozoid is a broad fibrous band, built up of many fibrils about 200 Å in diameter, which is associated with the nucleus and a spiral of modified mitochondria. Dense material around the basal bodies of the flagella runs along the fibrous band, so that the flagella are connected to the main skeletal structure of the cell. Complexity of organization is carried further in the cycad sperm, where Barton (1962) has found that some 25,000 flagella are arranged in a spiral tract which is underlain by a broad fibrous band. Within this band three layers of fibres could be recognized, the outermost of which is associated with a layer of dense material around the proximal ends of the basal bodies.

The uniflagellate *Trypanosoma* (Anderson, Saxe and Beams, 1956), *Leishmania* (Pyne, 1958; Pyne and Chakraborty, 1958) and *Blastocrithidia*, (Vickerman, 1962) and the biflagellate

Peranema and *Euglena* (Roth, 1958a, 1959) are other flagellates whose flagellar structure has been studied with the electron microscope. The basal structure of the uniflagellate forms is but poorly known, although Vickerman has shown that the basal body is complex and may have simple roots. In *Peranema*, the basal bodies of the two flagella lie at the inner end of the reservoir and Roth found a bundle of fibrils connecting the two flagellar bases. In the closely similar *Euglena*, fibrils were found to run from the flagellar bases to connect with the extensive system of subpellicular fibrils surrounding the reservoir and lining the body surface.

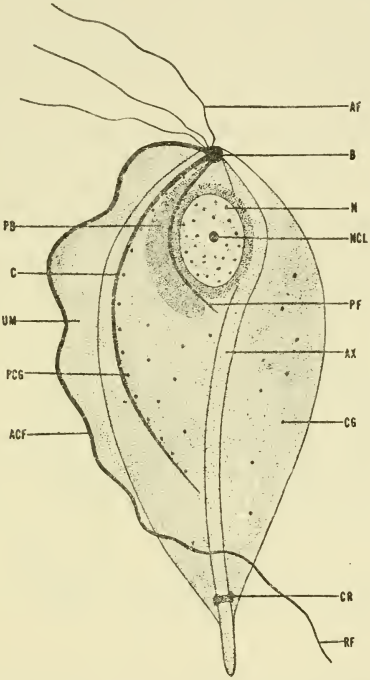


FIG. 16. Diagram of the structure of *Tritrichomonas* (=*Trichomonas*) *muris* to show the arrangement of the flagella and accessory structures (from Anderson and Beams, 1959). *AF*, anterior flagella; *B*, blepharoplast; *C*, costa; *PF*, parabasal filament; *AX*, axostyle; *RF*, recurrent flagellum; *ACF*, accessory filament; *UM*, undulating membrane.

Tritrichomonas carries three free anterior flagella and one recurrent flagellum which runs along the outer margin of the undulating membrane (Fig. 16). All four flagella arise in a blepharoplast region, in which Anderson and Beams (1959, 1961) found four normal basal bodies. From the base of the recurrent flagellum arises the costa, a coarse fibre about 0·4 μ in thickness which runs most of the length of the body and has obvious striations (period about 500 Å). A shorter and rather narrower striated fibre called the parabasal filament runs backwards from the basal body of an anterior flagellum to end just posterior to the nucleus. Both of these are probably supporting structures. A third large structure in this flagellate is the axostyle, a hyaline rod extending the length of the animal from near the blepharoplast complex to the posterior end; this may have a skeletal or contractile function.

The bases of the four or eight flagella of *Pyrsonympha* lie in the blepharoplast region at the anterior end of the body. A thick ribbon runs backwards from the blepharoplast-complex as a hook-shaped structure, and gives attachment to a very large number of tubular fibrils which are grouped to form the axostyle. In transverse section the axostyle shows a structure reminiscent of a " kinetodesma " of *Stentor*, since the fibrils are of similar appearance and dimensions, and are arranged in lamellae, 14 to 74 of which were found stacked together. Grassé (1956), who carried out this study, also noticed that the axostyle was contractile, and

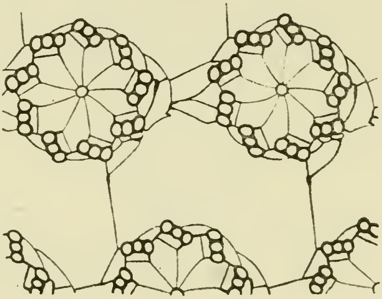


FIG. 17. Tentative diagram of the fine filament system in and around the proximal part of the basal bodies of flagella of *Trichonympha* (from Gibbons and Grimstone, 1960).

believes that its motion is independent of the flagellar movements because the structures do not move in unison. Note here that Randall and Jackson (1958) found that the " kinetodesmata " could well be the contractile structures at the body surface of *Stentor*.

In the more complex flagellates many more flagella may be present, e.g. *Lophomonas* has about 100 flagella (Beams, King, Tahmisian and Devine, 1958, 1960), while *Trichonympha campanula* has 12,000 to 14,000 (Gibbons and Grimstone, 1960), so that rather different root arrangements are to be expected. The flagella of *Lophomonas* arise around one end of the animal in concentric rows. Within the rows the basal bodies of the flagella are inter-connected by filaments at their inner ends and at the middle, giving a similar appearance to the connexions between the basal bodies of component cilia of cirri and membranelles.

The flagella of *Trichonympha* are borne in longitudinal rows, and the proximal regions of the basal bodies are regularly interconnected, both within and between the rows, by a complex of fine filaments about 20 Å thick (Fig. 17). Lying beneath the flagellar bases in the anterior region of *Trichonympha* is a rostal tube carrying striated ribs which continue in the posterior region as striated ribbons (striation period about 500 Å). These parabasal ribbons are fewer in number than the flagellar rows, and it is not certain if all the bases have connexions with the ribbons, although Pitelka and Schooley (1958) have found that the anterior ends of parabasal filaments are associated with flagellar bases.

More definite connexions are present between the basal bodies of the related flagellate *Holomastigotoides*, whose flagella are borne in spiral rows. Here the fine connexions may be lacking, but a thick fibrous band runs along the side of each row and connects all the bases together (Pl. XIIIa) (Gibbons and Grimstone, 1960). Incidentally, it is on the right of the row, in the position that the kinetodesma would occupy in a ciliate. The fibrous band is made of many very fine longitudinal filaments, with denser regions at each basal body and half-way between adjacent bases. Other dense bands of material are present beneath the flagella at the anterior end of the animal; these may be homologous with the striated fibres of *Trichonympha*.

6. Haptonemata

Several species of biflagellate algae of the genus *Chrysochromulina* (Chrysophyceae) (Parke, Manton and Clarke, 1955, 1956, 1958, 1959; Manton and Leedale, 1961) and the motile phase of a coccolithophorid known as *Crystallolithus hyalinus* (Parke and Adams, 1960), carry an appendage of much the same dimensions as a flagellum (Pl. IXd) which lacks the characteristic form of flagellar motility and may coil to the shape of a spring. Parke, Manton and Clarke (1955) have proposed the name haptonema for this structure, since it is thread-like and has an adhesive area.

In the small species the haptonema may be only about 5 μ long, but in *Chrysochromulina strobilus* lengths of up to 200 μ were reported; the diameter in all cases seems to be between 0·15 and 0·2 μ. Internally there are three concentric membranes, the outer one at least being similar in thickness to a ciliary membrane (Pl. IXe). These membranes surround a ring of longitudinal fibrils, 6 in some species, 7 or 8 in others. Each fibril appears tubular in transverse section, and has a diameter of 200 to 250 Å. No good evidence is yet available about the basal structure, which may give valuable information about the homology of these structures. All the haptonemata found have been extensible (and also contractile?) structures, which act as means of attachment, having either a clubbed adhesive tip, or an adhesive area along the whole length as in *C. strobilus*.

7. " Stereocilia "

Non-motile structures similar in appearance to normal cilia, but with reduced internal structure, have been described from mammalian epithelia by Wersäll (1956), Yasuzumi and Wakisaka (1956) and Nilsson (1957). These structures were first described from epididymal cells by Aigner (1900), but the name stereocilia was suggested by Reichel (1921). Aigner recognized that they were not true cilia, and Lucas (1932c) has summarized further evidence that they have different nature. The use of the electron microscope has demonstrated this conclusively, and Yasuzumi and Wakisaka found that stereocilia from rat epididymis were like long swollen microvilli, lacking internal fibrils.

Stereocilia found by Wersäll in the cristae ampullares of the guinea pig were about 40 μ long and about 0·2 μ thick. The single internal fibril extended only a short distance (about 4 μ) up the shaft of the structure, and also penetrated about 1 μ into the cytoplasm of the cell. These structures lack most of the characteristics of cilia, and in particular the basal body structure from which all true cilia originate, so that the name stereocilia should be abandoned for something more suitable.

The structures found in the rat oviduct by Nilsson were 3 to 5 μ long and 500 to 600 Å in diameter, with double outer membranes continuous with the cell membrane, and osmiophilic material internally, either in the form of a ring, or in the form of up to 6 fibrils. Occasionally these structures branch but the arrangement of fibrils at the branch was not seen, nor was any specialized structure seen in the basal cytoplasm.

8. Bacterial Flagella

Comparisons have sometimes been made between bacterial flagella and the flagella borne by animals and plants; these might be expected to share many features since both appear to be similar motile structures. Closer investigation reveals a number of important differences. Any attempts at comparison are complicated by the fact that all bacterial flagella are evidently not the same, and by the lack of detailed information about the structure of bacterial flagella. In addition, Pijper (1957) has reviewed the evidence for flagellar motility in bacteria and found it insufficient for proof that the flagella are self-motile organelles, in spite of the finding of Fleming, Voureka, Kramer and Hughes (1950) that the flagella start to move before the bacteria do, and that flagella may move on bacteria that are trapped and unable to move.

De Robertis and Franchi (1951) studied the flagella of *Bacillus brevis* under the electron microscope and found a central filament some 120 to 150 Å in diameter surrounded by a thin sheath. The central strand appeared to be a double helix of a trypsin resistant protein, with coiling of a variable pitch, while the sheath was dissolved by trypsin. A triple-threaded helix has been described by Starr and Williams (1952) in another motile bacillus. The basal structure is also inconsistent, and, as Pijper points out, it is not clear whether the flagella pass through the bacterial wall or not, and therefore whether they are attached to any form of basal body.

Small dense spherical bodies have been found at the base of flagella of *Proteus* (Houwink and Van Iterson, 1950).

On the basis of X-ray diffraction studies, Burge (1961) has presented two models of the arrangement of polypeptide chains within bacterial flagella. In one of these there are 3 filaments, each of 19 α-helices, and in the other—7 filaments, each of 7 α-helices. There is not sufficient evidence to decide between these two, but the latter model is favoured.

Astbury and Weibull (1949) found that the protein of bacterial flagella gave an X-ray diffraction pattern characteristic of the k-m-e-f group of proteins, i.e. it is in a group that contains both contractile and structural proteins. The amino acid composition of bacterial flagellar protein differs from that of true flagella in that the former is said to lack cystine and tryptophan (Tibbs, 1957).

It appears that no attempts should be made to draw inferences about true flagella from bacterial flagella, or vice versa, at least until we know more about the origin, structure and function of bacterial flagella.

CHAPTER 3

FACTORS WHICH AFFECT CILIARY ACTIVITY

1. Ciliary Activity and its Measurement

Contractions of either cilia or flagella in a fluid medium result in movements of the medium with respect to the organelle, although the form of beat shown by these two types of organelle and the water movements caused are rather different. Our knowledge of the effects of various factors on flagella (including sperm tails) is rather scanty, so that most of the information given in this chapter will concern the activity of cilia *sensu stricto.*

The rate at which fluids are moved by cilia must depend on a variety of anatomical and physiological characteristics of the organelle as well as on the effects of the environmental factors which are discussed in this chapter. Thus, the dimensions and compounding together of cilia are important, and so is the arrangement of cilia which are able to beat in a co-ordinated fashion, while the form and rate of beat of cilia and the mode of their co-ordination are fundamental in determining the effectiveness of the ciliary activity in the performance of a particular function. Ciliary co-ordination includes not only the characteristic ability of cilia to beat in a metachronal rhythm, but also the phenomena of reversal and inhibition, all of which are discussed in detail in Chapter V. It appears, then, that measurements of the mechanical work done by a group of cilia will not tell us much about the working of the ciliary mechanism, nor will an analysis of effects of many factors on any one part of ciliary activity be sufficient for an understanding of the complete system, for both the beating of cilia and their co-ordination are undoubtedly complex. It is the purpose of this chapter to review investigations on the modifications of ciliary activity caused by changes in the physical and chemical nature of the environment; only in a few cases is information available on both beating activity and co-ordination, which together make up the total ciliary activity.

Many methods of measuring the activity of cilia have been used during the last 100 years; most of these have been based on measurements of the rate of doing work by the cilia, while others depend on direct observations of the movements of the cilia. The earlier methods have been reviewed by Gray (1928), Lucas (1932a) and Nomura and Tomita (1933). Direct microscopic observation with a subjective estimate of the amount of movement, in such terms as " fast," " slow " or "stopped," is the first stage in most investigations, and almost all workers have moved on to estimate the mechanical activity of cilia. The most frequent method employed for this is the measurement of the speed of movement of particles of carmine, carbon or similar material (e.g. Gray, 1923, and many others), of a small platinum plate (Gray, 1923), or of small squares of filter paper (Parker, 1905) over the surface of a ciliated epithelium. Several workers have constructed more complicated devices in which they measured the rate of rotation of a small cylinder (usually of glass) which was held in contact with a ciliated epithelium (e.g. Engelmann, 1877; Inchley, 1921). In some cases it is possible to measure the rate of movement of water by cilia or flagella, as in some sponges (Parker, 1914), ascidians (Hecht, 1916) or kidney tubules (White, 1929). The rate of movement of spermatozoa and of small ciliated or flagellated organisms such as protozoa, rotifers, small flatworms and snails has sometimes been used, while in much the same class comes the method of Nomura and Tomita (1933) in which they measured the rate of crawl of a small piece of gill lamina of a lamellibranch mollusc.

Numerical data available by these methods are usually less valuable than measurements of the frequency of beat of the cilia. Few cilia beat slowly enough at normal temperatures for their movements to be followed by eye and counted, but two methods of observing faster cilia have been used with success. The stroboscope was used in studying the movement of cilia in the 1880's by several workers including Martius (1884); its employent was further exploited by Gray (1930), who gives a valuable account of the theory of this use of the stroboscope. This apparatus can be used only on cilia which beat more than 8 or 10 times a second, but is ideal for the examination of regularly beating cilia with a higher frequency of beat, and permits a quick and accurate measurement of the ciliary frequency.

Valuable results have been obtained with the cine camera by Gray (1930) and Lucas (1932a, b). This method has some advantages and some disadvantages as compared with the stroboscopic method. If the form of beat of a cilium is to be examined, the speed of the camera must be fast enough for at least ten frames to be exposed in each cycle of beat if an accurate picture is to be obtained. Rather slower speeds may be satisfactory for measurements of the frequency and examination of the metachromal waves. The stroboscopic method is valuable because the beating of the cilia can be seen throughout—by suitable adjustment of the speed of the stroboscope the cilia can be made to appear to beat very slowly, so that the form of beat can be examined and the metachronal waves can be studied. It is possible to photograph the "flash-images" seen in the stroboscope, and from the photographs to make a variety of measurements on the cilia and metachronal waves. For experiments on the frequency of beat and metachronism of faster cilia, the combination of stroboscope and single-exposure camera undoubtedly has an advantage over the cine camera in speed and ease, but the cine camera must be used in the examination of slower cilia. The examination of flagellar movement and sperm locomotion is usually very difficult, but a cine camera can sometimes be used.

These two methods both allow measurement of the actual frequency of ciliary beat. Where the metachronal waves can be clearly seen, the metachronal wavelength can be measured from the films, and the metachronal wave velocity can be calculated (wave velocity is frequency × wavelength). We have thus estimates of both the beating activity and the rate of metachronal wave transmission of the cilia. The amplitude of beat can be measured in some cases, so can the angular velocity of the effective stroke, and the speed of propagation of the bending wave along the cilium.

2. The Effect of Temperature on Ciliary Activity

The temperature varies quite widely in many environments, and it is usual to find that the rate of a biological process is altered by change in temperature. Ciliary activity is no exception, and both mechanical activity and the rate of beat are known to increase with increase in temperature. This does not necessarily mean, however, that cilia will beat faster in summer than in winter, for many factors combine to control the rate of ciliary activity; indeed, Usuki and Koizumi (1954) found that the ciliary activity of the gills of *Ostrea gigas* was at a maximum in winter and a minimum in summer, and they were able to correlate these changes with variations in the state of maturation of the gonads.

LABORATORY

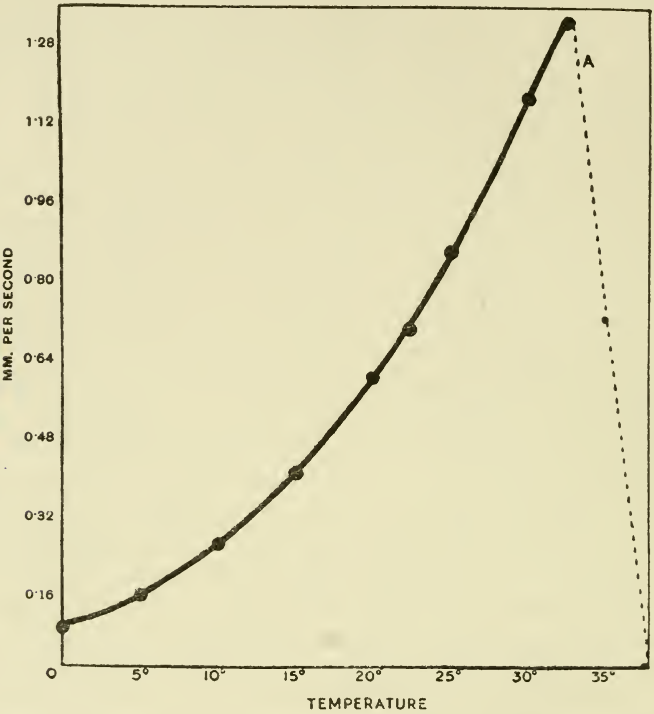


Fig. 18. The effect of temperature on the rate of movement of small particles by the frontal cilia of *Mytilus*. Observations shown here were made as soon as the tissue reached the required temperature. Activity fell away with time at temperatures of 30°C and above (from Gray, 1923).

The effect of temperature on the rate of movement of small particles by the frontal cilia of the gills of *Mytilus* was studied by Gray (1923), and his results are shown in Fig. 18. There is a gradual increase in the rate of ciliary activity over the range 0 to 35°C, although above about 32°C the amplitude of beat was found to be reduced. Further increase in temperature gave further reduction in amplitude followed by a reduction in the rate of beat of the cilia. *Mytilus* frontal cilia ceased to beat below 40°C, and at that temperature they were stationary in the relaxed position, while further heating to 45°C caused a change to the contracted position. Up to this temperature the changes were reversible, but at 47°C an irreversible change took place and the cytoplasm became opaque and coagulated.

Similar changes have been reported in the activity of the membranelles of *Stentor* (Sleigh, 1956a, b). The changes in frequency of beat, metachronal wavelength and metachronal wave velocity measured in an experiment on a row of these compound cilia are shown in Table 5. It is seen here that the wavelength changes with temperature, although not in a very regular manner. Aiello (1960) has found a similar change in wavelength in *Mytilus* cilia which was not detected by Gray (1930) or Lucas (1932b). The maximum rate of beat of *Stentor* membranelles occurs at about 30°C, above which the beat becomes intermittent and the frequency falls away. The metachronal wave velocity shows similar changes up to 36 to 38°C, at which temperature the metachronal co-ordination finally breaks down; the cilia cease beating below 40°C.

Secondary effects of high temperatures are found in the activity of both *Mytilus* and *Stentor* cilia at temperatures above 25 to 28°C. Below this temperature all changes of ciliary activity are rapidly reversible, indeed, in experiments on the effect of temperature on *Stentor*, the frequency was found to change to the level appropriate to its new temperature almost immediately (in a very few seconds at the most), and would then remain constant at that temperature for a long time. If the temperature was raised from say 20°C to 30°C and kept at the higher temperature, the frequency gradually fell away; on return to 20°C, the frequency was found to be below its original level and took some time to return to that level. The higher the temperature, the more marked are the secondary changes.

The primary effect of temperature on ciliary frequency and metachronal wave velocity may be compared by the application of the Arrhenius equation ($K = Ce^{-\mu/RT}$) to the results given in Table 5. This equation is more conveniently expressed as $\log K = C - \mu/RT$, where K is the rate of the process affected by temperature, C is a constant, μ is the temperature characteristic, R is the gas constant and T is the absolute temperature. A straight line will be obtained when log k is plotted against 1/T if the data fit the equation, and a temperature characteristic for these data can be calculated from the gradient of the line.

TABLE 5. THE EFFECT OF TEMPERATURE ON THE FREQUENCY OF BEAT, METACHRONAL WAVELENGTH AND METACHRONAL WAVE VELOCITY OF *Stentor* MEMBRANELLES.

Temperature (°C)	Frequency (beats/sec)	Metachronal wavelength (μ)	Metachronal wave velocity (μ/sec)
5·3	10·25	27·5	282
8·1	13·7	24·5	335
12·2	17·9	22·8	408
16·7	23·0	21·6	498
19·9	27·8	20·4	561
24·5	32·0	21·6	692
28·1	36·3	21·6	784
30·6	35·3	21·6	760
32·5	34·7	21·0	728
36·0	32·8	18·6	610

Graphs of this type, showing the effect on frequency and metachronal wave velocity of *Stentor* membranelles of temperatures in the range 0–25°C are given in Fig. 19. Values of μ calculated from a number of experiments on *Stentor* gave an average for frequency of 11,330 cal, and for wave velocity of 7520 cal; the difference between these two averages is significant.

The fact that both of these lines are straight suggests that we can expect the effect of temperature on both activities to be simple rather than complex. Gray (1923) found that the changes in activity of *Mytilus* frontal cilia corresponded very closely with changes in oxygen consumption caused by change in temperature, so that it appears that the rate of doing work is closely linked with the rate of respiration.

3. Viscosity of the Medium and Ciliary Activity

Viscous solutions have been used to slow up the movement of ciliated protozoans for the study of the living animals (see Marsland, 1943). By this means Pigon and Szarski (1955) found that the velocity of forward movement of *Paramecium* is inversely proportional to the relative viscosity of the medium (increased with dialysed gum arabic). It would be expected from Stokes' law that the movement of any body propelled by a constant force through a viscous medium would be slowed in this way; however, the retardation is the result not only of an increased resistance to the movement of the whole body, but also of a slowing of the ciliary beat.

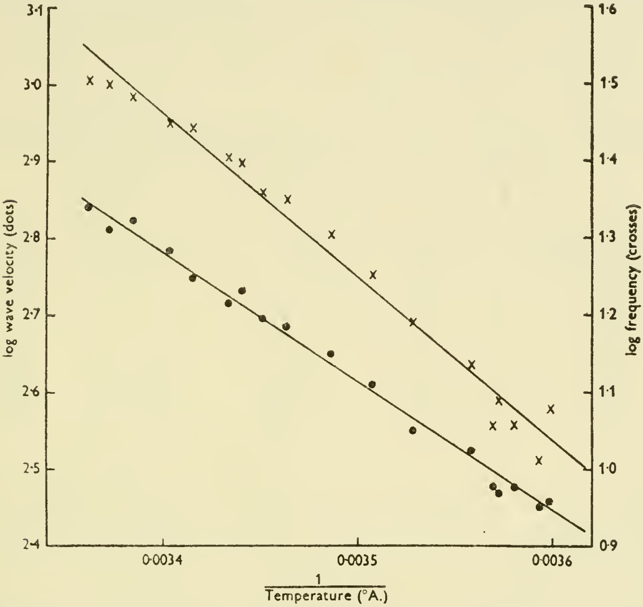


FIG. 19. The effect of temperature on metachronal wave velocity and frequency of beat of *Stentor* membranelles (see text) (from Sleigh, 1956a).

The effects of viscosity on the frequency and metachronal wave velocity of *Stentor* membranelles seem to be different at high viscosities from those found at lower viscosities, (i.e. up to about 3½ times the viscosity of water). Table 6 shows the changes of frequency, metachronal wavelength and metachronal wave velocity found in an experiment where the viscosity of the medium was increased with methyl cellulose in the range up to 3·56 cP. The primary effect of an increase in viscosity seems to be to slow the movements of the cilia through the medium, so that the frequency is decreased and the wavelength is increased, while the wave velocity remains unchanged.

Aiello (1960) has obtained similar results on the lateral cilia of *Mytilus* gills, although at higher viscosities the metachronal wave velocity shows a slight increase in average value. In experiments on the cilia of *Modiolus* and *Mytilus*, Gosselin (1958) used polyacrylamide as the viscous agent and found that viscosities up to 15 cP did not affect the frequency, or else stopped the beat completely, while the metachronal wavelength and metachronal wave velocity were markedly increased and the amplitude consistently reduced.

TABLE 6. THE EFFECT OF SMALL INCREASES IN VISCOSITY ON FREQUENCY, METACHRONAL WAVE VELOCITY AND METACHRONAL WAVELENGTH OF *Stentor* MEMBRANELLES (FROM SLEIGH, 1956A)

Methyl cellulose concentration (%)	Viscosity at 20°C (cP)	Frequency (beats/sec)	Wavelength (μ)	Wave velocity (μ/sec)
0	1·00	27·8	21·6	600
c. 0·1	1·35	27·1	22·4	606
c. 0·3	2·22	25·2	23·9	602
c. 0·5	3·56	22·8	26·1	595

The disagreement between these findings and the results quoted in Table 6 seems to have been solved by some experiments (Sleigh, 1961) on the effect of higher viscosities on *Stentor* membranelles. The results of these experiments are shown in Fig. 20. The changes of metachronal wave velocity do not differ significantly from zero at lower viscosities, while the frequency decreases steadily, until, at a viscosity of about 3·5 cP, both frequency and wave velocity curves turn upwards, indicating small decreases of frequency and very large increases of wave velocity of up to about 100 per cent. Since the frequency is slightly lower than normal, the wavelength is increased by over 100 per cent in some cases. Under these conditions of high viscosity the metachronal waves and the frequency are less stable than usual, and the amplitude is progressively reduced with increase of viscosity until the beats become a mere flicker. Metachronal waves cease and eventually all beating stops at about 10 cP.

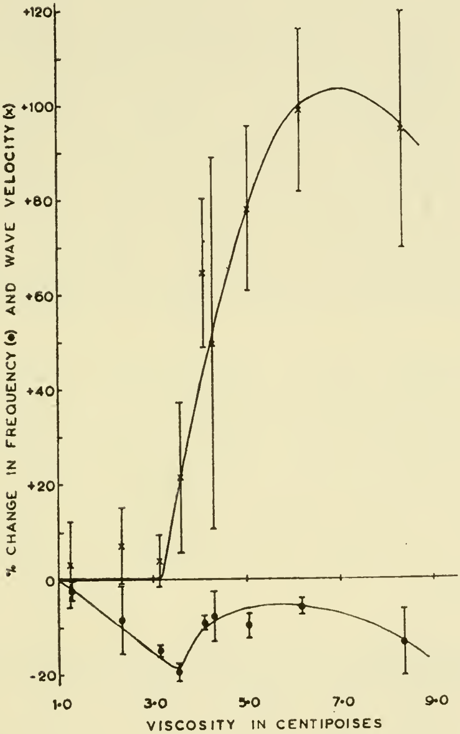


FIG. 20. The effect of viscosity on metachronal wave velocity and frequency of beat of *Stentor* membranelles. Each value plotted is the mean of many observations, the vertical lines showing 95 per cent confidence limits (from Sleigh, 1961).

The explanation given for these results is closely bound up with theories of the mechanism of metachronal co-ordination of these cilia which is considered in detail later (p. 184). At low viscosities the ciliary beat is slowed by the mechanical resistance of the viscous medium, while the metachronal co-ordination rate is unchanged because the conduction process is internal and independent of the frequency. As the viscosity increases, the movements of the viscous medium caused by the beating of any cilium have an increased effect on the movement of neighbouring cilia, and at about 3·5 cP the movement of one cilium begins to assist the movement of its neighbours sufficiently for the frequency to be increased. The direct communication of viscous drag from one cilium to the next is faster than the internal conduction process, and triggers off the contraction of the next cilium before the conducted impulse arrives, so that the metachronal wave velocity is increased. The higher the viscosity, the greater the viscous drag and the faster the metachronal wave velocity. The increases of both frequency and metachronal wave velocity are limited by the progressive decrease of amplitude, for with a small amplitude of beat less viscous drag is developed, neighbouring cilia assist each other less and eventually the metachronal waves break down.

A recent report that the metachronal wavelength and the metachronal wave velocity of *Opalina* cilia was decreased by an increase in viscosity (Potter, 1960) is very interesting, and has been confirmed by the author. The metachronal relationships of these cilia (p. 183) are quite different from those of *Stentor* membranelles, so that we need not necessarily expect the effect of viscosity to be the same; nevertheless, it is worthy of further experimentation.

4. Hydrostatic Pressure and Ciliary Activity

Many physiological processes have been studied under increased hydrostatic pressure, and valuable information has thereby been obtained. Our knowledge of the effects of pressure on ciliary activity is scanty. Regnard (1884) and Certes (1884) both found that pressures of 500–600 atm (6800–8000 lb/in^2) stopped the ciliary movement of various infusoria. Under increased pressure, *Paramecium* shows decreased speed of movement and sometimes complete stoppage, but Ebbecke (1935b) found that these changes were immediately and completely reversed on the release of pressure. Ebbecke's (1935a) experiments on *Beroe* swimming plates gave rather different results—their movements stop at about

150 atm (about 2000 lb/in²), but there was a strong acceleration of beat at about 200 atm, and the cilia finally stopped at 600 atm.

The effect of sudden changes of pressure on the frequency of beat of lateral cilia of excised gill filaments of *Mytilus* was studied by Pease and Kitching (1939). A sudden increase of pressure caused an immediate increase in frequency and a slow return to normal, while a sudden decrease of pressure caused an immediate decrease in frequency and a slow return to normal (Fig. 21). Within the range 1000 to 5000 or 6000 lb/in², the larger the change in pressure to which the cilia were subjected, the larger the frequency change. Above 5000 or 6000 lb/in² the ciliary frequency fell rapidly away and permanent injury was caused. Two effects of pressure on ciliary frequency are seen here; firstly, a deleterious effect of high pressures above about 5000 lb/in², which is common to other physiological processes, and secondly, a temporary change in the rate of beat occurring whenever the pressure is changed, which is comparable with a similar effect on cardiac rhythm (Edwards and Cattell, 1928, 1930).

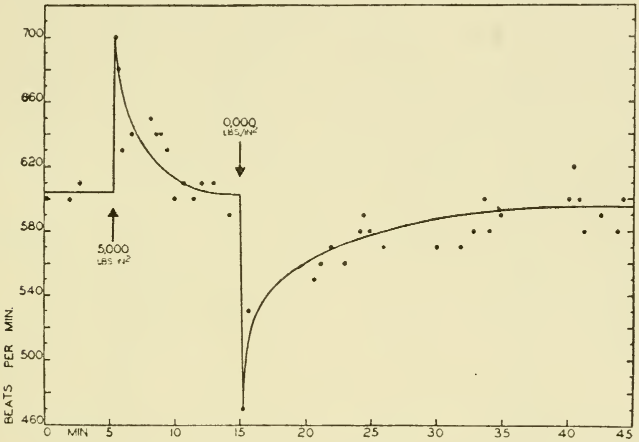


Fig. 21. The effect of sudden changes in hydrostatic pressure on the frequency of beat of lateral cilia of *Mytilus* gills (from Pease and Kitching, 1939).

Rather different results have been obtained in studies on the effect of increased hydrostatic pressure on the frequency of beat of cilia of protozoa, notably the membranelles of *Stentor* (Kitching, 1957). An immediate decrease in frequency was found to occur at pressures above about 1000 lb/in², and, although the beat persisted up to 10,000 lb/in² in some cases, metachronal rhythm usually broke down at about 4000 to 7000 lb/in². Investigations on *Stentor* by the present author (made using Dr. Kitching's apparatus, which he generously made available to me) have confirmed these findings on the effect of high pressure on the frequency of ciliary beat in this organism (Fig. 22). This effect is comparable with that found in *Paramecium* by Ebbecke. There is a slight but probably not significant increase in metachronal wave velocity at pressures up to 5000 lb/in². In addition to the

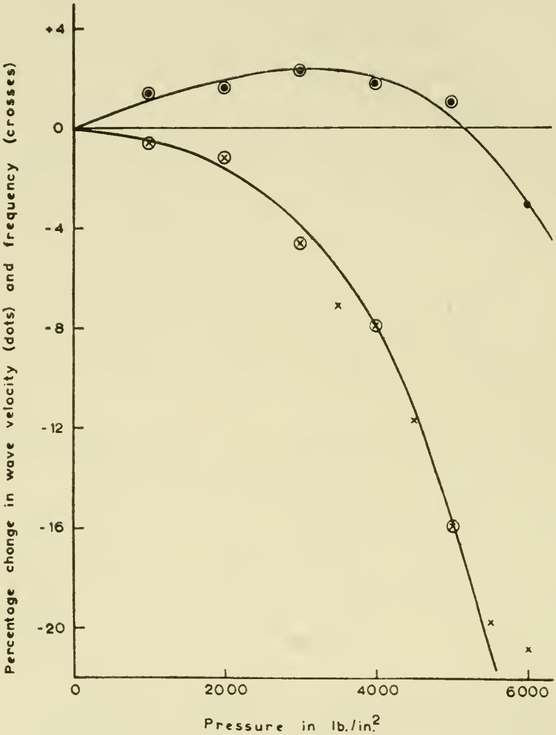


FIG. 22. The immediate effects of increased hydrostatic pressure on the metachronal wave velocity and the frequency of beat of *Stentor* membranelles.

immediate decrease in frequency, there was a further progressive decrease which was not immediately reversible; this increased with increase in time of exposure to high pressure, and was more rapid at higher pressures.

In observations on the effects of hydrostatic pressure on the activity of a wide variety of flagellates and ciliates, Kitching (1957) again found a difference in response in different animals. The movement of flagella or cilia of most of the organisms was reduced at high pressures, and such movement usually stopped after short exposure to pressures of 8000 to 14,000 lb/in^2; visible structural damage was found to occur at about the same pressure. An exception was found in the case of *Epistylis plicatilis* where pressures of the order of 1000 to 3000 lb/in^2 caused an increase in the rate of ciliary beat; higher pressures caused reduction of ciliary movement and eventually stoppage. Increased pressure was also found to affect the behaviour of many protozoa, for while some organisms, e.g. *Euplotes*, showed increased activity at moderate pressures (2000 to 4000 lb/in^2), others, e.g. *Stylonychia*, showed reduced activity. The ability to reverse and to perform avoiding reactions was lost in *Spirostomum* at pressures above about 4000 lb/in^2, and the ability of *Stylonychia* to perform normal controlled movements was lost at somewhat higher pressures. It is evident that systems of ciliary control are affected by these high pressures, and that effects on the contraction mechanism are not a complete answer to the problem.

It is impossible to determine at present the reason for the differences between the results obtained using different material. Further work should prove valuable, especially in cases where the contraction cycle of the cilia can be studied.

5. Electric Currents and Ciliary Activity

At about the turn of the century several workers experimented on the effects of electric currents on ciliated protozoa; experiments of this type on *Paramecium* have been reviewed by Wichterman (1953), and may be summarized as follows. When swimming paramecia are subjected to a weak direct current flowing between two electrodes in the surrounding medium, the animals will turn towards the cathode and swim towards it. If the current strength is increased more animals respond and swim rapidly towards the cathode. With a still stronger current, the rate of movement towards the cathode is first slowed, and then reversed so that the animals swim backwards towards the anode. Ultimately, the paramecia shorten and burst in a very strong current. Induction shocks have a similar effect.

An effect of electric current on the protoplasm of *Paramecium* was reported by Kinosita (1936). Paramecia which were stained with neutral red or nile blue sulphate and subjected to an electric current, showed a different colour at the two ends, giving an acid reaction at the cathodal end and an alkaline reaction at the anodal end. With increased current, the alkaline region spreads forward to cover the whole body.

It appears that the immediate cause of the changes in swimming direction has been found by Ludloff (1895), who noted that the direction of beat of the cilia on the surface of the animal nearest to the cathode was reversed when a current was passed between the electrodes. If the strength of current was increased, cilia over a larger area of the body showed a reversed beat. This effect could account not only for the reversal of movement in a strong current, but also for the orientation of the animal towards the cathode. Jahn (1960, 1961) has suggested that Ludloff's results are best explained by assuming that *Paramecium* is a "core conductor." He claims that by application of the laws of polarizing current he can explain the reversal of the ciliary beat and the activation of cilia of immobilized animals by the effects of the current on the membrane potential of the animal.

In relation to this last suggestion, several reports of work on *Opalina* by Japanese workers are very interesting. Okajima (1953) found that the direction of beat of the cilia in the area around the eletrode was changed by stimulation with a microelectrode placed on the body surface. The area of response depends on the strength of the current and on the position on the body. Currents of the order of 10^{-7} A were used here. Kinosita (1954) found that when he induced a temporary reversal of the ciliary beat with isotonic KC1, the cell membrane potential (measured with internal and external microelectrodes) suddenly fell to zero, with perhaps a transient overshoot. In fact, the membrane potential and direction of beat changed in close association. A reduction in membrane potential has also been found to accompany reversal of ciliary beating in *Paramecium* (Yamaguchi, 1960).

Naitoh (1958) has taken the investigations on *Opalina* further by using an intracellular electrode to apply an electric current. The effect of current intensity and direction on the membrane resistance is shown in Fig. 23. Membrane resistance is constant for inward current (i.e. internal electrode negative), but decreased to about one-tenth with an outward current of 4×10^{-9} A or more. In this example, the voltage applied when the current was zero was about 12 mV acting in opposition to the cell membrane potential; the average value at 13·7 to 16·2°C was 16 mV.

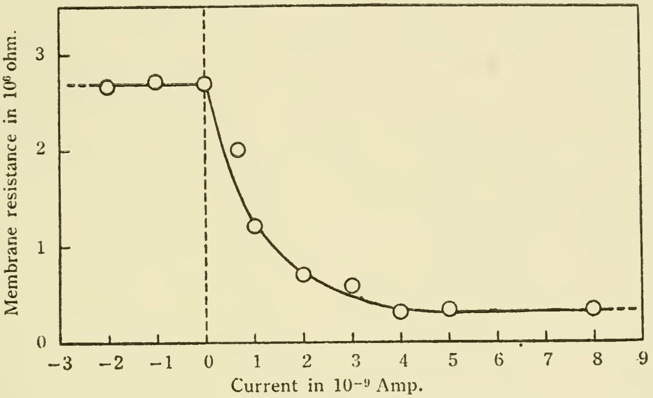


FIG. 23. The change in membrane resistance of the whole body of *Opalina* induced by application of direct current. Negative current values indicate inward current flow (i.e. the internal electrode was negative and the applied voltage greater than the membrane potential). Results measured at 15·5°C (from Naitoh, 1958).

Membrane resistance is not uniform over the whole of the cell surface, and topographical differences in ciliary reversal response may result from this.

Direct current stimulation with such a microelectrode, passing more than 2×10^{-10} A across the membrane in an outward direction, induced a change in direction of the effective stroke. If the current intensity is increased, the degree of change in direction is increased until the beat is completely reversed. When such a stimulating current is switched off, the beat in the normal direction is temporarily augmented. With inward current the

Table 7. The ciliary response in *Opalina* to transmembrane direct current of various intensities. The current was applied to the animal for about 10 sec at room temperatures between 12 and 25°c (From Naitoh, 1958)

Current strength (in 10^{-9} A)		Outward current	Inward current
Weak (0–10)	Make	Change in beating direction and perfect reversal (Metachronism is preserved)	Augmentation of normal stroke
	Break	Transient augmentation of normal stroke	Transient change in beating direction
Moderate (10–300)	Make	Perfect reversal of beating direction (Metachronism disappears)	Strong augmentation of normal stroke
	Break	Strong augmentation of normal stroke (continues for scores of sec)	Perfect reversal of beating direction (continues for scores of sec)
Strong (more than 400)	Make	Augmentation of normal stroke	Transient reversal of beating direction
	Break	Transient reversal of beating direction	Transient augmentation of normal stroke

normal beat is augmented, but is temporarily reversed when the current is switched off. Rather different results are obtained with higher current intensities, as can be seen from the observations quoted in Table 7. Naitoh found that it was possible to restore the ciliary beat of an animal that was dying, and had undetectable membrane potential, by the application of a suitable inward current. The restored beat was co-ordinated metachronally, and the animal survived for several minutes, provided that the application of inward current was continued.

It has long been known that potassium chloride would cause the reversal of *Opalina* cilia, and Naitoh found that the membrane potential of this animal is linearly related to the log potassium concentration at higher concentrations, although the slope of the curve decreases at concentrations below about 10 m mol. The resting potential of squid giant axons is related to potassium concentration in much the same way (Curtis and Cole, 1942). This suggests that a diffusion potential of potassium ions is the basis of the membrane potential in *Opalina*. Perhaps the change in potassium concentration changes the membrane conductivity, for the excitability (increase in current required to produce a given change in beat direction) is virtually constant over a wide range of potassium concentrations, and therefore over a wide range of membrane potentials. This finding led Naitoh to suggest that the induction of reversed beating of cilia is not directly related to the membrane potential. There can, however, be little doubt about the close connexion between ciliary reversal and the movements and concentrations of ions.

The movement of flagellates may also be affected by electric currents, and Mast (1927) found that the colonial flagellate *Volvox* swims towards the cathode when the organism is photopositive, while a photonegative organism swims towards the anode. These changes are due to the stoppage of flagellar movements on one side of the colony, which Mast explained on the basis of ionic movements. The difference in response between photonegative and photopositive *Volvox* seems to result from a difference in charge on the colony, photonegative colonies being positively charged and photopositive colonies negatively charged. The activity of flagella on the anodal side of positively charged colonies seems to be inhibited, so that the colony turns towards the anode and swims towards it; negatively charged colonies swim to the cathode. Mast suggested that an ion shift within the colony when it is an electric field could cause changes in potential and permeability, and increase in permeability causes decreased flagellar activity.

Electric currents are not without effect on ciliated tissues of metazoa, although this has been rarely studied. The beating of comb-plates of the ctenophore *Beroe* may be inhibited for ½ minute or more by passing a current of 1 to 2 mA/sq. cm through the animal from the oral (anode) to the aboral ends (Gothlin, 1929). Similarly, Segerdahl (1922) found that the laterofrontal cilia of the gill filaments of *Anodonta* were accelerated by a current of about 0·1 mA/sq. mm, while the lateral cilia are inhibited by this current, especially when the current is passing from the base of the ciliated cells towards their tips. She has also found that these lateral cilia were excited to a more rapid beat by passing a current of about 1 mA/sq. mm through the gill; after this current is switched off, ciliary movement may be inhibited in cells where the current had been passing from the surface to the base. It is interesting to compare these results on the lateral cilia with those of Naitoh on *Opalina*.

It is evident, then, that electric currents sometimes increase the movement of cilia, sometimes reverse it, and sometimes inhibit it, depending on the organism and the conditions. Attempts have also been made to detect the presence of electrical changes in ciliated tissues, and to correlate these with the ciliary activity. The change of membrane potential with reversed ciliary beat in *Opalina* has already been mentioned, and other electrical changes are also thought to take place.

6. Osmotic Pressure and Ciliary Activity

A slight increase in the osmotic pressure of the medium around the gill filaments of *Mytilus* may cause a slight reduction in the amplitude of beat, but no change in the rate of beat (Gray, 1922a). With a large increase in osmotic pressure all movement stops, irrespective of the nature of the osmotic agent. In hypotonic media, the gills of *Ostrea circumpincta* showed slightly increased mechanical activity at concentrations between 50 and 100 per cent sea water; below 50 per cent the activity was decreased and stopped completely at about 20 per cent sea water (Tomita, 1934a). Roots (1955) found that in two species of oligochaete worms the nephridiostome cilia were accelerated by dilution of the medium and slowed in hypertonic media, but these effects were only temporary. Large changes in the osmotic pressure of the medium caused some damage and serious long-term effects.

The effects of osmotic pressure on the change in beat direction of *Opalina* reported by Naitoh (1959) follow a rather similar pattern. In a hypertonic medium the animal shrank, but the direction of beat remained unchanged and the cilia continued to beat until the membrane was considerably deformed. On return to the normal medium, however, the beating quickly started again, but in the reversed direction, only to return to normal when the body had finished swelling. In a hypotonic medium, the swelling was accompanied by a reversal of the direction of beat, while the shrinkage on return to the normal medium had no effect on the direction of beat. Naitoh was able to show that these changes were due to hypotonicity and not to ion deficiency, and he deduced that the stretching of the membrane was the factor causing the change in the direction of beat of the cilia. Thus he could change the beat direction by stretching the cell membranes either by injecting liquids into the cell or by the use of two microneedles which were moved apart; in both cases the beat returned to normal on removal of the stimulus.

It is well known that the osmotic pressure of the medium affects the motility of spermatozoa, and here again it is found that hypotonic media have a greater inhibitory effect than hypertonic media. Emmens (1948) found that the effect of hypotonic media on rabbit spermatozoa was increased at acid pH and reduced in alkaline media.

7. Ions and Ciliary Activity

A reasonably steady balance of most ions is found in natural environments, but it is often useful to vary ionic concentrations experimentally in order to investigate the part they play in physiological processes. The concentration of hydrogen ions, on the other hand, varies quite widely in nature, and is found to have important effects on ciliary activity.

Gray (1920) reported that the gill cilia of *Mytilus* will not beat below pH 5·5 to 6·0, although the stoppage by acid is readily reversed on return to normal pH. Above a pH of about 9, the gill epithelium breaks up into its constituent cells, but the activity of the cilia does not stop. The effects of pH on the mechanical activity of the frontal cilia of *Mytilus* gill are shown in Fig. 24. However, all acids do not have an equal effect, for weak acids (especially carbonic acid) act more quickly than the strong mineral acids (Gray, 1922a). Thus Nomura (1932) found that the gill cilia of *Pecten* are stopped in 1 min at pH 6·15 in carbonic acid and at pH 3·7 in hydrochloric acid (at 13°C). Haywood (1925) and Tomita (1935) have reported similar findings in other lamellibranchs, and Jucci (1928) observed the same effect on cilia of the coelenterate *Sagartia*.

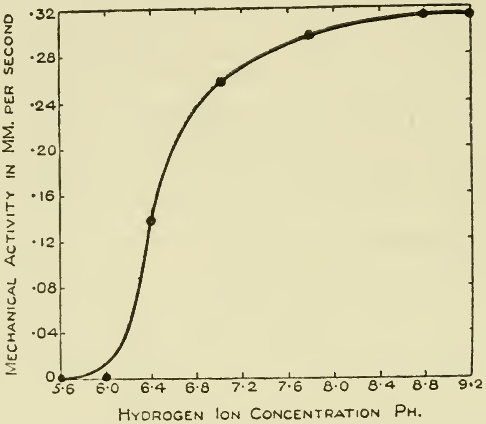


FIG. 24. The effect of pH on the mechanical activity of frontal cilia of *Mytilus* gill (from Gray, 1924).

The reason for this different effect of weak and strong acids is evidently concerned with the ease of penetration of the acids, and this in turn depends on their state of ionization. The penetration effect can be illustrated by Gray's (1924) experiments on the relation of ciliary activity to oxygen consumption. Oxygen consumption and ciliary activity are closely related; in the presence of either weak or strong acids the ciliary beat is stopped and the oxygen consumption falls, but it is only completely abolished if the acid is able to penetrate the whole cell, and thus only the weak acids are fully effective. Gray (1928) has suggested that the stoppage of cilia of molluscs like *Mytilus* by carbonic acid is important in the life of these animals because the accumulation of CO_2 in the closed shell inhibits the ciliary movement and reduces the oxygen consumption. Nomura (1932) denies this and suggests that the stoppage of cilia in an enclosed space results from oxygen lack, since the amount of CO_2 is not sufficient for stoppage. It is quite possible that neither explanation is correct, and that the stoppage of the beat of at least some gill cilia is a nervous reflex action (see p. 121).

All cilia do not give the same response at the same pH, and it seems that there is some adaption to the normal pH of the surroundings. Thus, Tomita (1934b) reported that the gill cilia of *Ostrea circumpincta* are more resistant to pH than those of *Mytilus*. The cilia of different internal regions of the body of *Mya* normally function at different pH, and Yonge (1925) found that a different pH was required to stop the movement of cilia of different origins; these findings are shown in Table 8.

TABLE 8. THE RELATION BETWEEN THE MINIMUM pH AT WHICH CILIA FROM DIFFERENT CILIATED EPITHELIA OF *Mya* WILL FUNCTION AND THE NORMAL pH OF THEIR SURROUNDINGS (FROM YONGE, 1925)

Origin of cilia	Minimum pH at which they can function	Average pH of the fluid normally around them
Style sac	3·5 – 4·0	4·45
Stomach	4·0	5·8
Mid-gut	4·4 – 4·8	6·2
Oesophagus	4·4 – 4·8	6·6
Rectum	4·4 – 4·8	6·9
Gill	5·2 – 5·8	7·2

Cilia of *Paramecium* cause maximum forward movement at pH between 5·1 and 6·3 (Chase and Glaser, 1930; Dryl, 1961); activity falls away quickly in more acid media and slowly in alkaline media. The activity of spermatozoa varies with pH in much the same way. In a study of rabbit spermatozoa, Emmens (1947) found that sperm are immobilized and quickly die at pH below 5·8, but retain motility for several hours at pH up to 9·5 or 10; alkali may be used to restore motility to spermatozoa stopped by acid, provided the exposure to acid is short.

The difficulties of studying the effects of other ions on ciliary activity have been emphasized by Gray (1928). Many ionic changes upset the stability of the ciliated epithelium and may lead to breakdown of the intercellular matrix, so that only changes which affect the ciliary mechanism without damage to the epithelium are of interest here. Also, most media contain a complex balance of ions, and it is difficult to make changes which do not upset the whole balance. Thus it is not enough simply to remove one type of ion completely, for this may upset the osmotic equilibrium, while, if one ion is replaced by another, it is difficult to decide whether the changes which result are caused by the lack of one ion or the excess of the other. Many of the earlier workers were not sufficiently cautious about these things, e.g. Gray (1928) attributes most of the effects of anions on ciliated tissues reported by Lillie (1904) to effects on tissue stability and not on the cilia themselves.

Ciliary activity of *Mytilus* gill is largely unaffected by changes in the concentration of anions, provided that the normal balance of cations is maintained in the medium. Gray (1922b) made up artificial sea waters in which the normal balance of Na^+, K^+, Ca^{++}, and Mg^{++}, was provided, but in which only one anion was present. Frontal cilia of *Mytilus* gills beat normally in such media containing only one of the following: Cl^-, NO_3^-, I^-, Br^-, CH_3COO^-, and SO_4^{--}, but not in tartrate and citrate. The divalent metals form unionized salts with these last two anions, so that they effectively destroy the cation balance of the medium. The cilia of protozoa seem to show a similar indifference to the relative concentrations of anions.

Studies on the effects of cations on protozoan cilia have mainly centred around the reversal of the ciliary beat of the body cilia of some ciliates; we have already seen (p. 90) that this is closely bound up with membrane potential. Merton (1923) found that a sudden increase in the concentrations of Na^+ or K^+ induced reversed swimming in *Stentor*, while similar increases of Ca^{++} or Mg^{++} did not. Investigations on *Paramecium* by Mast and Nadler (1926) and Oliphant (1938) both showed that the monovalent cations K^+, Na^+, Li^+ and NH_4^+ generally induced reversal, while the divalent and trivalent cations did not. The former authors, who used a large number of chemicals, found that the polyvalent cations were able to neutralize monovalent ones, and they concluded that cation unbalance upset the electrical equilibrium of the membrane.

The concentration of ions present in the medium may modify the reversal response of these protozoan cilia. Okajima (1954b) found that if *Opalina* is placed in a calcium-free Ringer solution, it quickly loses the ability to reverse the direction of beat in response to a stimulus; the same happens in potassium chloride isotonic with the normal medium, where the absence of Ca^{++} is again likely to be the cause. At other potassium concentrations there is little change in the threshold of the reversal response, although the duration of reversal is longest at medium concentration. The higher the concentration of calcium in the medium,

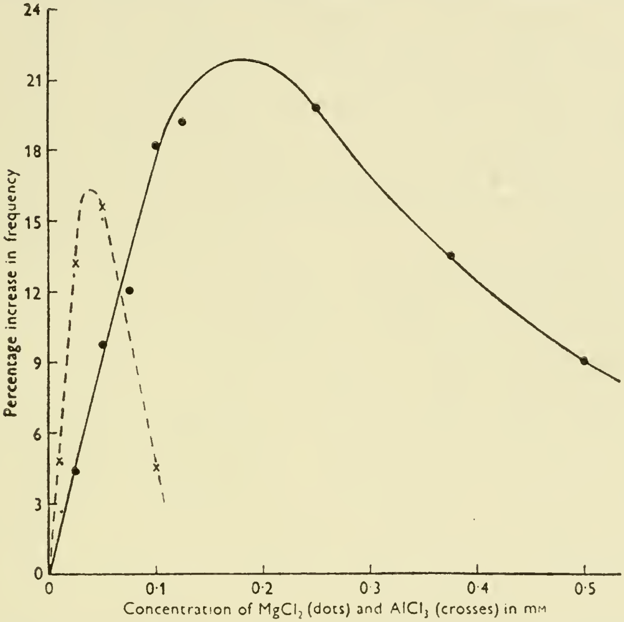


FIG. 25. The effect of magnesium chloride and aluminium chloride at various concentrations on the frequency of beat of *Stentor* membranelles (from Sleigh, 1956a).

the more marked is the reversal response, both in its longer duration and in its lower threshold level. An accumulation of Ca^{++} in the cell seems to be necessary if the cilia are to show reversal, although Ueda (1956) has shown that a maintained reversal is evoked in *Opalina* injected with Ca^{++} precipitants, so that reversal is evoked by a diminution of the free Ca^{++} in the cell.

Some polyvalent ions have been found to increase the beating activity of the membranelles of *Stentor* in experiments on animals cultured in a medium containing only K^+, Na^+, Ca^{++}, Cl^-, HCO_3^- and PO_4^{---} (Sleigh, 1956a). The effects on the frequency of beat of *Stentor* membranelles of the addition of magnesium chloride and aluminium chloride to this medium are shown in Fig. 25. A maximum increase in frequency of beat of about 20 per cent was recorded at a magnesium chloride concentration of 0·125 m mol, the addition of which increases the total molarity of the medium by less than 10 per cent, but markedly increases the ratio of divalent cations to monovalent cations (about 4:150 in the normal medium). Aluminium chloride increases the rate of beat at even lower concentations. It is interesting that these increases in the rate of beat were not accompanied by any change in the metachronal wave velocity. In this report it was suggested that the effects of Mg^{++} and Al^{+++} on the beating activity were the result of changes in protoplasmic viscosity caused by the action of the ions on the ionisation of protoplasmic colloids, since Heilbrunn (1923) had found that the addition of magnesium chloride reduced the viscosity of *Stentor* protoplasm. It is also possible that Mg^{++} may affect the chemical parts of the contraction and energy liberation processes, in which it is known to participate, as well as interfering with the normal balance between polyvalent and monovalent cations.

The effect of cation concentrations on metazoan cilia has been mainly studied on *Mytilus* gill cilia, where the effects on different groups of cilia are not always the same. Among the monovalent cations, K^+ and Na^+ are the most abundant, and both can be varied over a wide range without affecting the beat of the frontal cilia. In balanced solutions of calcium chloride, magnesium chloride and the chloride of a single monovalent cation, Gray (1922b) found that the frontal cilia were stopped in Li^+ and accelerated in NH_4^+ and K^+, especially the latter, while in Na^+

the beat continued at the normal rate. He therefore placed these ions in the series $Li^+ < Na^+ < NH_4^+ < K^+$, the order of their effect on the ciliary beat.

In general it seems that increase in the concentration of K^+ leads to increase in the rate of beat of *Mytilus* cilia. Not only do the frontal cilia beat fastest in salts of this metal, but the laterofrontal cilia also beat faster and with reduced amplitude in solutions with increased concentration of K^+. The lateral cilia of excised gills normally lie quiescent in sea water, an effect which Gray (1926) has attributed to the large concentration of Mg^{++} in sea water as compared with that in the blood. However, K^+ seems to be antagonistic to Mg^{++}, and an increased K^+ concentration results in prolonged activity of the lateral cilia. Kinosita (1952) reported that isotonic potassium chloride produces a sudden increase in the angular velocity and frequency of beat of the abfrontal cilia of *Mytilus* gills.

The divalent ions Ca^{++} and Mg^{++} are important, and lack of either of them leads to irreversible changes in the functioning of the cilia of *Mytilus*, even if they are replaced by other divalent cations. Changes in the total amount of polyvalent cations upsets the balance between monovalent and polyvalent ions, which is normally fairly steady in any particular environment. An increased concentration of Ca^{++} has little effect on the ciliary activity of *Mytilus*, but in the absence of Ca^{++} the ciliary beat gradually slows down and eventually ceases, although it slowly recovers on return to the normal medium. Gray (1924) has suggested that the absence of calcium upsets the supply of energy to the cilia, particularly the conversion of chemical energy to mechanical energy, without affecting the consumption of oxygen. It is now known that small quantities of both Ca^{++} and Mg^{++} are important for the liberation of energy in the reaction breaking down ATP to ADP.

Wide variations of Mg^{++} concentration have little effect on the rate of beat of frontal cilia of the *Mytilus* gill, but the stability of the intercellular matrix is rapidly affected by lack of Mg^{++}. The effect on *Mytilus* gill lateral cilia is rather different, for these cilia beat most rapidly in a medium from which Mg^{++} is absent, achieving eventually a fast beat with small amplitude. In higher concentrations of Mg^{++}, even that found in normal sea water, the lateral cilia are soon brought to rest. Gray (1926) believes that the concentration of Mg^{++} in the blood is more important than that of the surrounding sea water in the determination of the activity of these lateral cilia. It is difficult to see why the concentration of Mg^{++} should affect one group of cilia differently from another, but, if the beat of lateral cilia is normally controlled by the presence of a substance such as serotonin (p. 110), then the Mg^{++} may interfere with the action of this substance.

8. Oxygen, Respiration and Ciliary Activity

In the absence of oxygen, cilia of *Mytilus* gills were found by Gray (1924) to gradually slow down and stop, but they would quickly recover in aerated water if the oxygen lack was not prolonged; the lateral cilia of the gill stop well before the frontal cilia (Aiello, 1960). Similarly, the application of cyanide very quickly reduces the oxygen consumption of *Mytilus* tissues, and the ciliary activity falls away more slowly (Gray). In an earlier paper Gray (1923) had reported that, in *Mytilus*, the rate of movement of a small platinum plate over the gill surface was directly proportional to the rate of oxygen consumption of the gill tissues, when the ciliary activity was changed by changes in temperature. He also found (Gray, 1924) that when lateral cilia of *Mytilus* gill which were quiescent were reactivated by the addition of veratrine, K^+ or a mixture of K^+ and OH^-, the commencement of activity was accompanied by an increase of between 40 and 50 per cent in the oxygen consumption. It appears then that oxygen is essential for the activity of these cilia, but that they will function anaerobically for a short time in much the same way as many other tissues. Usuki (1956a) and Usuki and Okamura (1956) have found that both aerobic and anaerobic processes are important for the ciliary activity of the oyster gill; some energy may be derived from the anaerobic part of the respiratory sequence.

Many spermatozoa may be stored under conditions of low oxygen supply and are capable of either aerobic or anaerobic respiration, although normally there is a close relation between sperm motility and the rate of respiration (Mann, 1954). Similarly, some ciliated and flagellated protozoa live in situations that are almost if not completely without oxygen, e.g. the flagellate parasites of the termite gut (Cleveland, 1925). Kitching (1939a, b) has found that cilia of free-living ciliated protozoa ceased to beat in the absence of oxygen, and that cilia of the peritrichs *Cothurnia*, *Vorticella* and *Zoothamnium* stopped within 5 to 15 min, while those of *Paramecium* continued to beat for an hour or more.

The close link between respiratory activity and the movements of cilia and flagella established by these studies is to be expected in view of the constant supply of energy required for these movements. It is also not surprising that cilia and flagella may be used in anaerobic conditions by organisms which have been able to release energy for other vital activities in the absence of oxygen.

The route of supply of energy from the reserve substances to the ciliary mechanism is now fairly well known. Spermatozoa of both vertebrates, e.g. bull (Mann, 1954), and invertebrates, e.g. sea urchin (Rothschild and Cleland, 1952; Mohri, 1957a), seem to carry phospholipids as an energy store, and, in the absence of other available substrates, these are broken down to provide energy for movement. Carbohydrate is present in mammalian semen, however, and this is broken down (although usually only anaerobically to lactic acid) to provide energy for sperm motility in preference to the phospholipid stored in the sperm. Glycogen seems to form the energy store in the gills of the oyster, and Usuki and Koizumi (1954) have found that seasonal changes in ciliary activity and changes in the glycogen content of the gills show similar trends. The most important substrates in ciliated tissues are probably carbohydrate, in spite of the fact that Gray (1924) found a respiratory quotient of 0·84 in *Mytilus* gill. Both carbohydrate and phospholipid can be broken down by processes involving the tricarboxylic acid cycle after preliminary stages which are different for the two substrates. The main stages in these degradations are shown in Figs. 26-28.

Observations which confirm that the breakdown of these substrates by this route provides the energy required for the activity of cilia and flagella may be summarized as follows:

(i) Monoiodoacetic acid inhibits the activity of cilia of the oyster gill (Usuki, 1956a), lateral cilia of *Mytilus* gill (Aiello, 1960) and sperm (Mann, 1954). It blocks stage 7 (and also stage 23), and results in the accumulation of glyceraldehyde-3-phosphate in oyster gill tissue (Usuki and Okamura, 1956).

(ii) Sodium fluoride, which inhibits the activity of cilia of oyster gills (Usuki, 1956a) and reduces the respiration and activity of sperm (Mann, 1954), blocks stage 10 so that an accumulation of 3-phosphoglyceric acid occurs in oyster gill

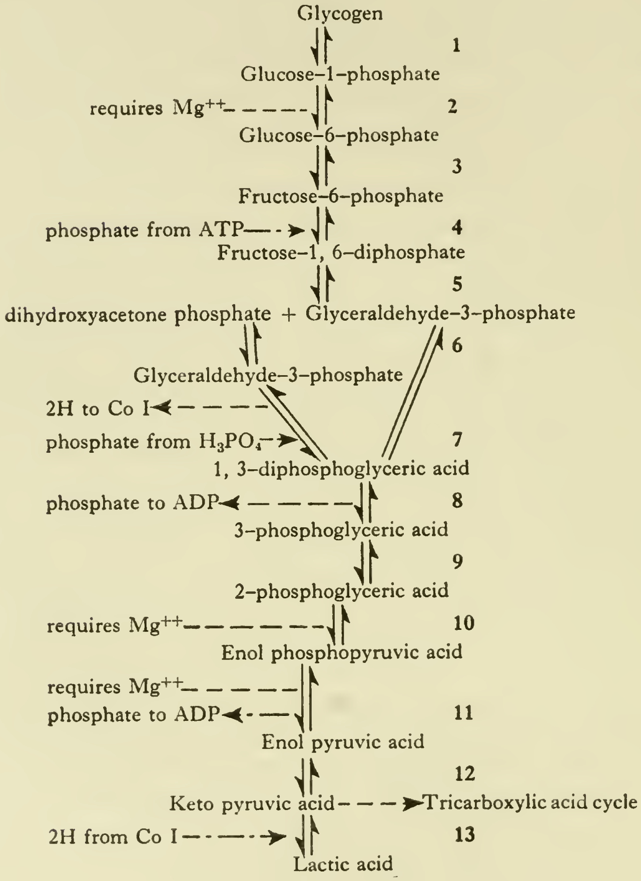


FIG. 26. The breakdown of glycogen in glycolysis to provide pyruvic acid for the tricarboxylic acid cycle (Fig. 27). Reference is made in the text to the stages in this sequence of reactions by the numbers given in this figure.

tissue (Usuki and Okamura, 1956). The effect of fluoride, which forms a complex with magnesium and phosphate, may be reduced by the addition of pyruvate or succinate, but not by the addition of glucose.

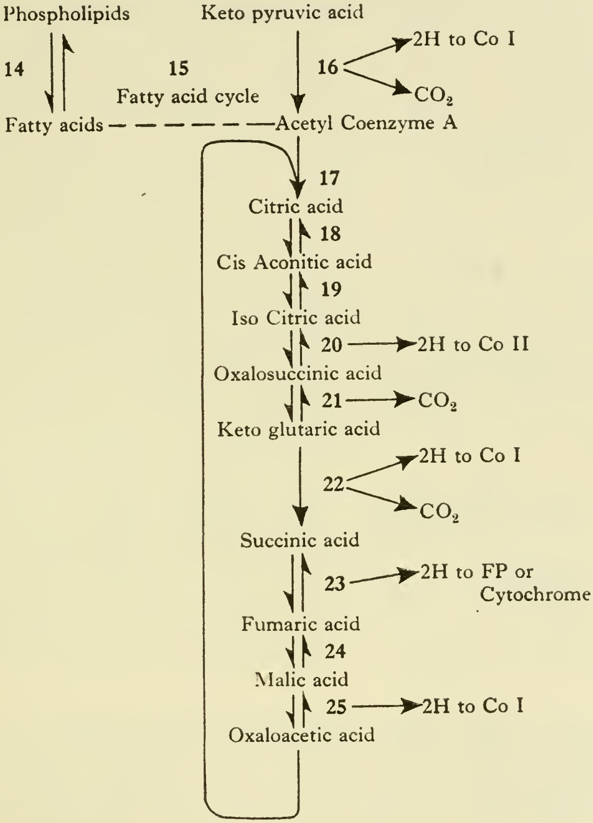


FIG. 27. The citric acid or tricarboxylic acid cycle. The stages shown are referred to in the text by the numbers given in this figure.

(iii) Sodium azide and 2-4-dinitrophenol (DNP) affect phosphorylation reactions such as stage 4, and treatment with both azide and DNP led to an accumulation of fructose-6-phosphate in the tissues of oyster gills (Usuki and Okamura, 1956); in DNP there was also a reduction in the concentration of fructose-1-6-diphosphate below the normal level. Azide inhibits the ciliary activity of oyster gills (Usuki, 1956b) and has been shown to reduce both the frequency and the metachronal wave velocity of lateral cilia of *Mytilus* gills to about one-third of their normal values at a concentration of 0·01 mol (Aiello, 1960). DNP increases the oxygen consumption, but depresses ciliary activity of *Mytilus* gills (Weller and Ronkin, 1952; Aiello, 1960) and inhibits the ciliary activity of oyster gills (Usuki, 1956c). Both DNP and azide reduce sperm activity (Mann, 1954).

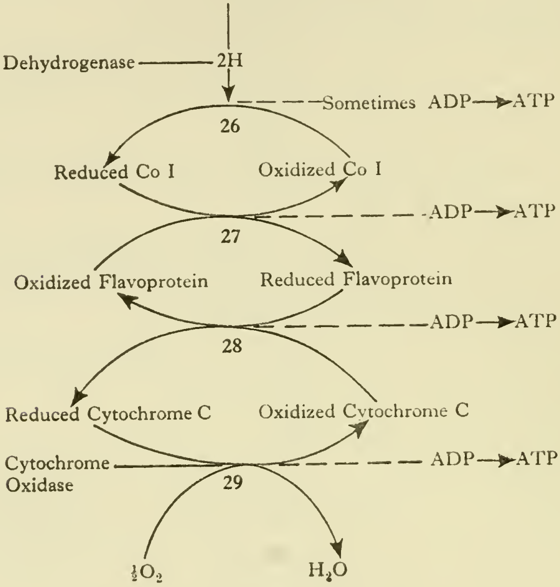


FIG. 28. The coupling of oxidation to phosphorylation. The dehydrogenase enzymes may transfer hydrogen through coenzymes (Co I or Co II in Figs. 26 and 27) or directly to later stages in the chain shown. Numbers of stages are again used for reference in the text.

(iv) Malonate (sodium salt or acid) reduces the ciliary activity of oyster gills (Usuki, 1956c), sperm motility (Mann, 1954) and the oxygen consumption of the sea-urchin sperm (Mohri, 1957b). It blocks stage 23, but stimulates stage 25, so that Mohri found that the total dehydrogenase activity may be increased in malonate, particularly if malate is added.

(v) Sodium cyanide reduces the respiration and ciliary activity of *Mytilus* gill (Gray, 1924; Aiello, 1960) and reduces the activity of oyster gill cilia (Usuki, 1956b) and spermatozoa (Mann, 1954). It prevents the action of cytochrome oxidase and blocks stage 29. In oyster gill tissue, Usuki and Okamura (1956) found some accumulation of pyruvic and lactic acids in the presence of cyanide.

(vi) Fluoroacetate, which blocks stage 18, was found to reduce the oxygen consumption of sea urchin sperm (Mohri, 1957c).

(vii) Chloral hydrate, which probably blocks stage 7, has been shown to reduce the rate of respiration of *Mytilus* gill tissue slightly and to markedly reduce the activity of the gill cilia (Gray, 1924). It appears that chloral hydrate may have a secondary effect on the ciliary mechanism.

There is thus abundant evidence that the respiration of ciliated tissues normally involves both anaerobic glycolysis, the tricarboxylic acid cycle and the cytochrome system. The part played by adenosine triphosphate (ATP) as a short-term energy store and an energy carrier between these respiratory processes and the ciliary mechanism is discussed in the next section.

9. Adenosine Triphosphate and Ciliary Activity

It seems well established that ATP provides the immediate source of energy for the beating activity of cilia in much the same way as it does for the contraction of muscle. Much of the evidence for this is derived from the use of " models " of cilia and flagella, but some direct evidence is available.

Vorhaus and Deyrup (1953) found that the activity of cilia from the pharyngeal mucosa of the frog could be increased by up to 100 per cent by the addition of ATP at a concentration of $1 \cdot 5 \times 10^{-5}$ mol, while smaller increases in activity were obtained at higher and lower concentrations. Hydrolysis of the ATP destroys the effect on ciliary activity. These results have been confirmed by Usuki (1959), who, using similar material from the frog as well as ciliated epithelia from the gills and palps of the oyster, obtained an acceleration of ciliary activity at ATP concentrations between $3 \cdot 3 \times 10^{-7}$ and $3 \cdot 3 \times 10^{-4}$ mol; there was also an increase in the secretion of mucus. Usuki also used a histochemical method which enabled him to demonstrate the presence of ATP-ase in the cilia and epithelial cells of the oyster and the frog, and the mucus-secreting cells of the oyster. The ATP-ase may be located in the actual ciliary shafts, for isolated cilia of the protozoon *Tetrahymena* possess enzymatic activity capable of splitting ATP, ADP and A-5-MP (Child, 1958). An extract of flagella of *Polytoma* was found by Tibbs (1957) to split ATP, ADP, AMP and beta-glycerophosphate (see also p. 29). Lansing and Lamy (1961b) have used a technique that shows localization of ATP-ase activity as a deposit of lead; in electron micrographs of the cilia of the rotifer *Philodina* the metallic deposit appeared in well-defined regions outside peripheral fibrils 1 and 5 of the ciliary shafts.

ATP also acts as the energy carrier for the movement of spermatozoa. It has been found in both ram sperm (Mann, 1945) and sea urchin sperm (Rothschild and Mann, 1950), and perch sperm contain an ATP-ase (Tibbs, 1959). *Mytilus* sperm tails have been shown to contain an ATP-ase whose activity could be increased by addition of magnesium chloride, and to a lesser extent by addition of calcium chloride (Nelson, 1955). The ATP-ase activity of the sea urchin sperm was found by Mohri (1958) to be mainly located in the tail, with a small proportion in the mid-piece. Using biochemical data obtained from bull sperm Nelson, (1958) has calculated that more energy than that required for movement could be liberated by succinic dehydrogenase activity (i.e. through the respiratory activities outlined above) and carried by the ATP present; the necessary enzymes seem to be present in the tail, and Nelson believes them to be located in the outer nine fibrils. ATP-ase activity in bull sperm seems to be associated with a myosin-like component that Bishop (1958d) has found in the sperm tails; this confirms the similarity of the relationship between ATP and cilia to that between ATP and muscle. These two relationships are also similar in that the action of sperm tail ATP-ase is dependent on the concentration of Mg^{++} and Ca^{++} and can be inhibited by salyrgan.

Models of cilia and flagella have been prepared by the glycerine-extraction techniques pioneered by Szent Györgyi (1949) and Weber (1953) in work on the part played by ATP in muscle contraction. Glycerine-extracted models of flagella of spermatozoa and trypanosomes made by Hoffmann-Berling (1954, 1955) were found to show a spontaneous alternation of contraction and relaxation when ATP was added. This beating activity was suppressed by small quantities of ATP-ase inhibitors like salyrgan (5×10^{-5}) and germanin (2×10^{-6}). Similar models of sperm of the squid and various mammals have been prepared by Bishop (1958a, c, f) and by Bishop and Hoffmann-Berling (1959). They all responded to ATP, and the mammalian sperm models also contracted with ADP and inosine triphosphate (ITP), but not with pyrophosphate or other inorganic ions. In all cases the response was a rhythmic flagellation with a similar frequency and amplitude to that in fresh sperm. In this more recent work, no correlation was found between ATP concentration and either frequency or amplitude, although Hoffmann-Berling (1954) believed that ATP concentration controlled the frequency of contraction.

Alexandrov and Arronet (1956) found that cilia of the frog palate epithelium which had been extracted with glycerine for several days were immobile until ATP was added, but then burst into activity, with a rate of beat which was often similar to that of normal cilia. Rhythmic contraction occurs even when the cilia are isolated from the basal protoplasm. The reactivated cilia sometimes beat synchronously, but the normal metachronal co-ordination was lost. Cilia of *Vorticella* could also be reactivated with ATP after being frozen and dried (Levine, 1959).

Perhaps the most interesting thing about these reactivated models of cilia and flagella is that they show repeated contractions, while muscle models only show a single contraction; there is relaxation of the cilia models but not of muscle models. It seems that the ability to perform alternate contractions and relaxations must be built into the contractile mechanism of these organelles, for much of the cilium structure, including the cell membrane with its special properties of selective permeability, is destroyed in the preparation of the models. Hoffman-Berling (1955) noted that ATP may be serving a double function, acting specifically as an energy source for contraction, and unspecifically as a plasticizer for relaxation; he attributed the effect of ATP concentration on rate of beat that he found to its plasticizer action. Pyrophosphate may also act as a plasticizer, for Bishop (1958a) has found that addition of pyrophosphate with ATP may increase the amplitude of contraction. In addition, Bishop (1958e) has demonstrated a Marsh-factor type of relaxing system in sperm tails similar to that known in muscle; this is sensitive to Ca^{++}, since models with ATP and Marsh-factor from rabbit muscle would not contract until Ca^{++} was added.

It is important to notice that although contractions occur alternately on the two sides of the cilia or flagella models, the contractions are not propagated along the organelles as bending waves, and there was no forward movement or spiralling of sperm models.

10. Hormones and Ciliary Activity

Several substances known to act as hormones and chemical mediators in other parts of animals have been found in ciliated epithelia, and have been shown to have effects on the activity of cilia. It is not easy to be certain whether these substances are acting directly on the ciliary mechanism, and in no case is the role of the hormones fully understood. A summary of results obtained with hormone substances mentioned is given in Table 9.

Perhaps the most interesting of these substances is **serotonin** (**5-hydroxytryptamine**), which is believed to be released by some motor nerves of molluscs, and has been found to excite the hearts of some crustaceans and some molluscs at very low concentrations (Welsh, 1954: Page, 1958). It is also known to increase the activity of rhythmically contracting smooth muscle fibres of the chick amnion, at a stage when no nerve connexions are present (e.g. see references in Page, 1958), and to increase the rate of beat of isolated mammalian atria (Trendelenberg, 1960). Recently, a fairly wide interest has been taken in this substance, both in vertebrates and invertebrates, but its action does not seem to be understood in any of the many places in which it has been found.

TABLE 9. THE EFFECTS OF SOME HORMONE SUBSTANCES ON CILIARY ACTIVITIES. CONCENTRATIONS ARE GIVEN IN G/ML

Drug used	Concns. causing increase	Maximum increase		Concns. causing decrease	Maximum decrease		Material	Reference
		of	at		of	at		
Frequency of beat								
Serotonin	10^{-7}–10^{-5}	93 %	10^{-5}				*Mytilus*	Aiello (1960)
	10^{-9}–10^{-4}	150 %	10^{-7}				*Mytilus*	Gosselin (1961)
ACh	10^{-7}	10%	10^{-7}	10^{-6}–10^{-3}	23 %	10^{-3}	*Mytilus*	Bülbring *et al.* (1953)
Eserine	10^{-6}	3 %	10^{-6}	10^{-5}–10^{-3}	16 %	10^{-3}	*Mytilus*	Bülbring *et al.* (1953)
				10^{-3}	25 %	10^{-3}	*Mytilus*	Aiello (1960)
Adrenaline	10^{-6}–10^{-5}	14 %	10^{-5}				*Mytilus*	Bülbring *et al.* (1953)
	10^{-8}–10^{-5}	6 %	10^{-7}				*Stentor*	Sleigh (1956b)
Particle transport								
ACh	10^{-6}	11 %	10^{-6}	10^{-5}–10^{-3}	48 %	10^{-3}	*Mytilus*	Bülbring *et al.* (1953)
	10^{-5}	27 %	10^{-5}	10^{-4}	33 %	10^{-4}	*Rana*	Kordik *et al.* (1952)
				10^{-5}–10^{-4}			*Metridium*	ten Cate *et al.* (1955)
Eserine	10^{-5}–10^{-3}	33 %	10^{-4}	10^{-2}	28 %	10^{-2}	*Mytilus*	Bülbring *et al.* (1953)
	10^{-5}–10^{-4}	95 %	10^{-4}	10^{-3}	11 %	10^{-3}	*Rana*	Kordik *et al.* (1952)
Adrenaline	10^{-6}–10^{-4}	76 %	10^{-4}				*Mytilus*	Bülbring *et al.* (1953)
	10^{-5}	25 %	10^{-5}				*Rana*	Plattner and Hou (1931)
	10^{-6}–10^{-5}						*Metridium*	ten Cate *et al.* (1955)
Metachronal wave velocity								
Serotonin	10^{-5}	105 %	10^{-5}				*Mytilus*	Aiello (1960)
Eserine				10^{-3}	10%	10^{-3}	*Mytilus*	Aiello (1960)
Adrenaline	10^{-8}–10^{-5}	17 %	10^{-5}				*Stentor*	Sleigh (1956b)

Aiello (1957, 1960) and Gosselin (1961) have found that serotonin accelerates both the frequency of beat and the metachronal wave velocity of lateral cilia of *Mytilus* gill, e.g. by about 100 per cent at a concentration of 2×10^{-5} mol (Aiello). By assay using the clam heart, it was estimated that 1 g (wet weight) of gill tissue possessed an activity equivalent to 0·4 to 7·6 μg of serotonin. Gosselin and Ernst (1958), who used photofluorimetric analysis to estimate the serotonin concentration in lamellibranch gill tissue found values between 0·7 and 1·0 μg/g (wet weight), which agree well with the result obtained by Aiello. These authors obtained a prompt and sustained acceleration of the beat of lamellibranch gill cilia, which was graded over a wide concentration range from as little as 10^{-10} mol serotonin; they also obtained an acceleration of beat with known antagonists of serotonin like tryptamine, 5-hydroxytryptophan, dihydroergotamine, LSD and brom-LSD. Further confirmation of the presence and activity of serotonin has been provided by Blaschko and Milton (1960), who found that serotonin is oxidised in homogenates of *Mytilus* gill tissue, so that a system is available for the removal of the substance after it has acted on the ciliary mechanism.

The tentative suggestion that serotonin may be released by, or by the action of, the branchial nerve of *Mytilus*, which runs near the gill axis, has been put forward by Aiello (1960). It is possible that it is carried in the blood spaces from the nerve endings to the gill filaments, where it acts on the ciliary mechanism. More work on this substance should provide exciting results, although no very marked response has been obtained in experiments on the membranelles of *Stentor*, and preliminary reports of other investigations do not agree.

Many more attempts have been made to find a relationship between **acetyl choline** (ACh) and ciliary activity. Some thirty years ago Plattner and Hou (1931) and Ishikawa and Ohzono (1931) reported that the activity of cilia of the frog's pharynx was increased by ACh. The latter authors found that the action of ACh is prevented by atropine, and suggested that the parasympathetic nervous system is concerned with the control of these cilia, while the former authors believed that the tissues lack ninervation and that the ACh was acting directly on the ciliary mechanism. The stimulant effect of ACh on ciliary activity of the frog pharynx has been confirmed by Kordik, Bülbring and Burn (1952), who found that lower concentrations (10^{-5} g/ml) of both ACh and eserine (an anticholinesterase) increased the rate of particle transport by the cilia, while higher concentrations of both depressed the activity. Similar results were obtained with ciliated epithelium from the rabbit trachea by these authors, and in both tissues atropine slowed the activity. There is no certainty in these cases that ACh does not act on nerve tissues which could influence the ciliary activity.

In an attempt to use a ciliated tissue free from nerve and muscle, Bülbring, Burn and Shelley (1953) experimented on the frontal cilia of *Mytilus* gill filaments. It is unfortunate that these gill filaments have been shown to contain both muscle and gland cells (Lucas, 1931a). In their experiments Bülbring, Burn and Shelley found an increase in both the rate of particle transport (11 per cent at 10^{-6} g/ml) and the rate of beat (10 per cent at 10^{-7} g/ml) with ACh bromide, while both activities were slowed at higher concentrations. Similarly, eserine sulphate increased the rate of particle transport over a wide range of concentrations, and only at a very high concentration (5×10^{-3} g/ml and above) did it depress this activity; this substance had little effect on the rate of beat except for some slowing at the higher concentrations. Atropine gave much the same results as ACh, instead of the opposite effect that one would expect, but *d*-tubocurarine reduced ciliary activity at all concentrations, as it should do if it antagonizes ACh. The effect of ACh on particle transport seems to be mainly the result of changes in the rate of beat, for Aiello (1960) found that while both eserine and a mixture of eserine and ACh slowed the frequency, they did not affect the metachronal wave velocity.

Bülbring, Burn and Shelley have been able to show the presence of ACh in normal gill filaments by both pharmacological assay and paper chromatography, and a cholinesterase enzyme, which is necessary for the breakdown of ACh, was also present. These authors had some difficulty in finding a choline acetylase system for the synthesis of ACh, but its presence in the gill filaments of *Mytilus* has been confirmed by Milton (1959). It would be most interesting if it could be proved that the ACh is associated with the ciliated cells rather than the muscle or gland cells, and that the action of ACh on the cilia was a primary and not a secondary effect.

Experiments on the effect of ACh on ciliary movement have also been carried out on some other animals. For example, in coelenterates the cilia of the tentacles of *Metridium* were slowed by ACh (10^{-4} and 10^{-5} g/ml) (ten Cate, Coomans and Walop, 1955), while in protozoa, both flagellates and ciliates have been shown to have acetyl cholinesterase activity. Bülbring, Lourie and Pardoe (1949) estimated that *Trypanosoma rhodesiense* contained 2·4 to 8·6 μg of ACh/g (wet weight) of tissue, and demonstrated *in vitro* synthesis of ACh using trypanosome material; they could neither detect nor obtain synthesis of ACh in the non-flagellate *Plasmodium.* In the ciliate *Tetrahymena pyriformis* Seaman (1950) and Seaman and Houlihan (1951) have shown the presence of an ACh-esterase which is inhibited by eserine (10^{-7}) and diisopropylfluorophosphate (DFP) (10^{-7} mol). The movement of living *Tetrahymena* is reversibly inhibited by both eserine and DFP at a concentration of 4×10^{-3} mol; this seems rather a high concentration, but it does not affect the rate of glycolysis of the cells. Seaman (1951) has further demonstrated by differential centrifugation that the ACh-esterase activity is located in the fibrillar system of the pellicle. Although Tibbs (1960) was able to demonstrate ACh-esterase activity in sperm of the trout and perch, he could not detect any activity in homogenates of *Polytoma uvella*, *Polytomella caeca* or *Tetrahymena pyriformis.*

The effect of ACh on these protozoan cilia may not be very specific, for it has also been found to affect the reversal response in *Paramecium.* Müller and Tóth (1959) found that both ACh bromide and eserine sulphate affect the duration of the reversal response caused by the addition of $\frac{1}{60}$ mol KCl. At low concentrations of ACh (10^{-4}) and eserine (10^{-5} and 10^{-6}) the duration of reversal of the ciliary beat was lengthened by up to 200 per cent, while at higher concentrations the response did not last as long as in the control animals. Bayer and Wense (1936a) have extracted ACh from *Paramecium.*

Evidence for a direct effect of ACh on ciliary beating is not very convincing in spite of all these reports. The effect of adrenaline, and also of serotonin in molluscs, is larger and more consistent, and it is quite possible that the changes in ciliary activity caused by ACh may be but secondary results of the action of ACh on some other parts of the ciliated cells, or even on other cells of the organism.

Rather less work has been done on the effects of **adrenaline** on ciliary activity, but the results obtained show good agreement. The activity of the ciliated epithelium of the frog pharynx was found to be increased by 20 to 25 per cent in adrenaline (10^{-5} g/ml) by Plattner and Hou (1931), and adrenaline also increased the activity of cilia of the mucous membrane of the rabbit trachea (Kordik, Bülbring and Burn, 1952). Invertebrate tissues show similar results, e.g. adrenaline concentrations of 10^{-5} and 10^{-6} g/ml were found to accelerate the activity of cilia on the tentacles of *Metridium* (ten Cate, Coomans and Walop, 1955). Both the rate of ciliary beat (max. of 24 per cent at 10^{-5} g/ml) and the rate of particle transport (max. 138 per cent at 10^{-4} g/ml) by the cilia of *Mytilus* gills were increased by adrenaline at all the concentrations used by Bülbring, Burn and Shelley (1953). In protozoa, Bayer and Wense (1936b) extracted a substance from *Paramecium* which they believed to be adrenaline and which had an adrenaline-like action on the contraction of rabbit intestine.

Adrenaline consistently accelerates ciliary activity in all the metazoan tissues mentioned above at all of the concentrations used; this shows an important difference from ACh which accelerates ciliary activity at low concentrations and depresses it at higher concentrations. In the presence of adrenaline at concentrations as small as 10^{-8} to 10^{-5} g/ml, both the frequency of beat and the metachronal wave velocity of *Stentor* membranelles were increased, particularly the wave velocity, which was increased by about 27 per cent in one experiment at 7×10^{-6} g/ml (Sleigh, 1956b). It appears therefore as if the adrenaline acts on a part of the system common to both the contraction and conduction activities. This shows a parallel with heart muscle, where both the rate of beat and the rate of conduction may be increased by adrenaline (Clark, 1927; Prosser, 1950). Perhaps the finding of Goffart and Brown (1947) that the action of adrenaline on striated muscle is closely connected with the potassium concentrations on either side of the muscle membrane may be found to be important for cilia also.

Noradrenaline had no effect on the ciliary activity of the rabbit trachea (Kordik, Bülbring and Burn, 1952), but accelerates the activity of cilia on the tentacles of *Metridium* (ten Cate, Coomans and Walop, 1955).

11. Effects of Other Chemicals on Ciliary Activity

(*a*) *Alkaloids.* It has long been known that **veratrine** influences the activity of cilia. Gray (1924) found that the lateral cilia of *Mytilus* gill filaments could be activated by veratrine, or by increasing the concentration of potassium ions, the two being mutually antagonistic. More recently, Aiello (1960) has come to the conclusion that veratrine and potassium ions may actually augment each other, since at a particular potassium concentration an addition of veratrine increases the ciliary activity, and at a particular veratrine concentration an addition of potassium increases the ciliary activity. In normally active lateral cilia Aiello found that the addition of veratrine sulphate produced marked increases in both the frequency of beat and the metachronal wave velocity, as shown in Table 10.

TABLE 10. THE EFFECT OF VERATRINE SULPHATE ON THE ACTIVITY OF LATERAL CILIA OF *Mytilus* GILLS. (FROM AIELLO, 1960) (COPYRIGHT 1960 BY THE UNIVERSITY OF CHICAGO)

Medium	Number of observations	Frequency (beats/sec)	Wavelength (μ)	Wave velocity (μ/sec)
Sea water	12	12·3 (1·5)	11·0 (1·6)	134 (21)
0·0005% VS	4	11·5 (2·6)	12·1 (1·6)	140 (46)
0·001% VS	12	19·8 (4·3)	10·3 (2·4)	204 (39)
0·005% VS	6	21·6 (2·2)	11·3 (0·6)	244 (27)
0·01% VS	10	19·3 (4·3)	11·0 (1·9)	213 (39)

Figures in brackets are standard deviations.

Gray (1928) suggested that the veratrine acts on the respiratory processes which provide the energy for ciliary movement, as had also been postulated for muscle. However, Aiello found that the respiratory rate and ciliary activity always changed together and in the same way under the influence of veratrine, either alone or in combination with respiratory inhibitors; he was not able to show

TABLE 11. THE EFFECTS OF VARIOUS ALKALOIDS, GLYCOSIDES AND OTHER SUBSTANCES ON CILIARY ACTIVITY

Substance	Increase	Decrease	No effect	Material	Reference
Alkaloids—					
Veratrine	PT			*Mytilus*	Gray (1924)
	F, MWV			*Mytilus*	Aiello (1960)
	PT			*Rana*	Ishikawa and Ohzono (1931)
Strychnine	PT			*Rana*	Ishikawa and Ohzono (1931)
			MA	*Metridium*	ten Cate *et al.* (1955)
Pilocarpine	PT			*Rana*	Ishikawa and Ohzono (1931)
	PT			*Rana*	Plattner and Hou (1931)
Nicotine		F		*Mytilus*	Aiello (1960)
		F		*Rana*	Liaci (1940)
			MA	*Metridium*	ten Cate *et al.* (1955)
Cocaine		PT		*Rana*	Ishikawa and Ohzono (1931)
			PT	*Rana*	Kordik *et al.* (1952)
Aconitine	PT			*Rana*	Ishikawa and Ohzono (1931)
Emetine	PT			*Rana*	Ishikawa and Ohzono (1931)
Quinine		PT		*Rana*	Ishikawa and Ohzono (1931)
Caffeine		PT		*Rana*	Ishikawa and Ohzono (1931)
Atropine			PT	*Rana*	Ishikawa and Ohzono (1931)
	PT*	PT*		*Mytilus*	Bülbring *et al.* (1953)
		F		*Mytilus*	Aiello (1960)
Tubocurarine		PT, F		*Mytilus*	Bülbring *et al.* (1953)
Arecholine	PT			*Rana*	Plattner and Hou (1931)
Physostigmine (eserine)	F, PT*	F, PT*		*Mytilus*	Bülbring *et al.* (1953)
Glycosides—					
Strophanthin	PT			*Rana*	Ishikawa and Ohzono (1931)
Digitoxin	F, MWV			*Stentor*	Sleigh (1956a)
Digitamine	PT			*Rana*	Ishikawa and Ohzono (1931)
Digitalin		F, MWV		*Mytilus*	Aiello (1960)
Others—					
Methacholine		MA		*Metridium*	ten Cate *et al.* (1955)
Camphor	PT			*Rana*	Ishikawa and Ohzono (1931)

PT, particle transport; F, frequency; MWV, metachronal wave velocity; MA, mechanical activity; * indicates an increase at low concentration and decrease at high concentration.

an independent effect of veratrine on respiration. If veratrine were to increase the rate of ciliary activity, this would of course result in an increase in the rate of respiration. High concentrations of veratrine stop the cilia in the contracted position, and this contracted state can only be broken by the removal of veratrine or interruption of the energy supply. It does not seem very likely that an excess of available energy should result in such a continuous contraction, although energy may be necessary for the maintenance of the contraction.

In veratrine treated muscle, a single stimulus results in a prolonged contraction, apparently because of an increase in the duration of the excitatory state. It seems likely that veratrine acts on the excitation part of the ciliary mechanism, since it affects both frequency and metachronal wave velocity. From recent work on muscle excitation (e.g. Kiebel and Sandow, 1957; Hoffmann, Feigen and Genther, 1962) it appears that the veratrine may be concerned with ionic conditions across the cell membrane; older work on veratrine and ciliary activity suggests that potassium ions may be important here.

Several alkaloids, including veratrine, strychnine, aconitine, pilocarpine and emetine have been found to increase the activity of cilia of the frog pharyngeal epithelium, while cocaine, quinine and caffeine depress the activity of these cilia (Ishikawa and Ohzono, 1931). The effects of a number of alkaloids on ciliary activity are summarized in Table 11.

(*b*) *Digitalis glycosides.* The activity of *Stentor* membranelles is stimulated by **digitoxin** (Sleigh, 1956a). This glycoside was found to increase both the frequency of beat and the metachronal wave velocity as shown in Fig. 29. The increase in wave velocity was much larger than that in frequency, and showed a maximum at a lower concentration, so that it appears that the effects of digitoxin on wave velocity and frequency may concern different parts of the ciliary mechanism. It has been suggested that digitoxin acts on cardiac muscle by a lowering of the threshold of excitability (Sollman, 1950), and the same explanation could be used here. There is some evidence that glycosides may influence permeability to ions.

Aiello (1960) has reported that another digitalis preparation, digitalin, reduced both the frequency (by 24 per cent) and the metachronal wave velocity (by 48·5 per cent) of *Mytilus* lateral cilia at a concentration of 2×10^{-5} mol. Yet another preparation digitamin, has been found to cause a slight increase in the ciliary activity of frog pharyngeal epithelium (Ishikawa and Ohzono, 1931). A summary of the effects of these glycosides, and also of strophanthin (ouabain) which has a digitalis-like action on the heart and accelerates the activity of frog pharynx cilia, is given in Table 11.

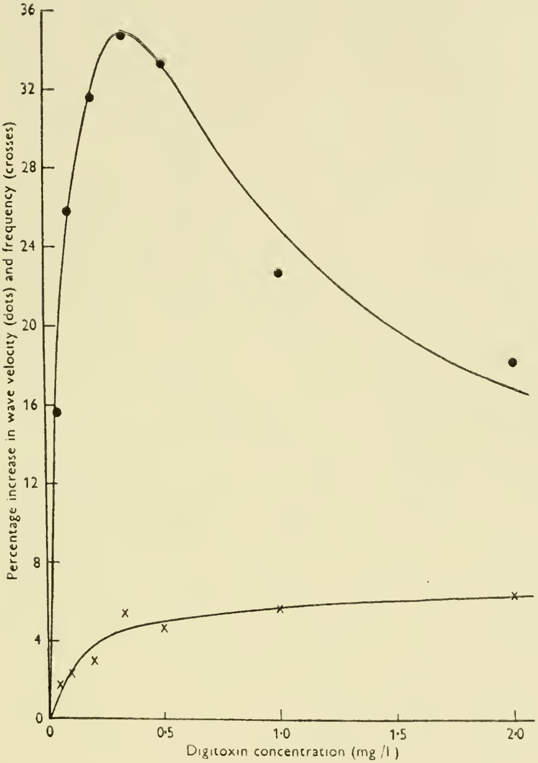


FIG. 29. The effect of digitoxin concentration on the frequency of beat and the metachronal wave velocity of *Stentor* membranelles (from Sleigh, 1956a).

(*c*) *Miscellaneous chemicals.* Several substances which affect the action of cilia by their interference with the energy supply to the cilia have been mentioned earlier, as have the effects of acids, alkalis and ions. The effects of methacholine (methyl ACh) and camphor are shown in Table 11. Camphor seems to act directly on the ciliary mechanism, according to Ishikawa and Ohzono (1931), who found that the acceleration effect of ACh was inhibited by atropine, but if camphor was then added the cilia were again accelerated. Rajapurkar and Panjwani (1961) have found that diacetylmonoxime depresses the activity of oesophagus cilia of the frog at concentrations above 10^{-8} g/ml.

12. Control of Ciliary Activity by the Organism

Three types of ciliary organization have been described by Gray (1928) on the basis of the way in which the cilia may be controlled. Firstly, there are cilia which appear to lack any form of external control and are normally in a state of continuous activity. Secondly, some cilia may be controlled by activation; these are normally inactive or beating feebly and are controlled by excitatory stimuli. Lastly, many cilia may be under inhibitory control; these cilia are normally active, but may be stopped by inhibitory stimuli. The inhibitory or excitatory control is usually independent of any metachronal co-ordination, since it may spread over an entire ciliated area almost instantaneously, travelling much faster than the metachronal waves. Where control exists, it influences the whole activity of the group of cilia, so that inhibition stops the movement of all the cilia, while activation may increase both the rate of beat and the rate of metachronal co-ordination.

It is not surprising that organisms have found it necessary to control cilia which are used for locomotion and some other vital activities. In protozoa the body cilia of a ciliate like *Paramecium* appear to be in continuous movement, but two examples of control are well known. When *Paramecium* performs an avoiding reaction, as a result of a chemical or physical stimulus, for example, the cilia are reversed for a few moments and then return to their normal beating activity. The activity of the main body ciliature may thus be modified in response to a stimulus. Jennings (1931) has also described thigmotactic inhibition of cilia of *Paramecium*, in which all the body cilia may cease activity when the animal comes to rest in contact with some solid body. The membranelles of *Stentor* appear to stop under some inhibitory control, for these compound cilia cease to beat as soon as the animal contracts, and also stop their normal activity when the animal swims backwards.

Control by activation also seems to be present in protozoa. The cirri of hypotrichs like *Euplotes* and the long stout cilia of *Cyclidium* show sudden activation of the cilia resulting in rapid movements by co-ordinated action, although these cilia are normally quiescent. The co-ordination shown in these movements is not metachronal co-ordination, although a similar mechanism may be involved. Activation of the cirri of *Euplotes* may be accompanied by inhibition of the membranelles which is abolished by cutting the fibril which connects the " motorium " to the membranelles (Taylor, 1920).

Even among the simpler flagellates like *Astasia* and *Euglena*, the movement of the flagellum may be modified by stimulation of the organism. Borgers and Kitching (1956) found that a change in the carbon dioxide tension caused *Astasia* to change its direction of movement, while Kitching (1961) reports that a sudden decrease in the carbon dioxide tension caused this flagellate to cease its forward movement and perform repeated turns before settling to forward motion again. Similarly, in the well-known light responses of *Euglena*, there must be a change in the flagellar activity in response to a change in the direction or strength of illumination.

The presence of a well-developed nervous system in metazoan animals allows a means of controlling the activity of cilia, and it appears that both excitatory and inhibitory control are frequently used. It is difficult to be certain whether there are any metazoan cilia which are without any form of control, though there may be some cilia with cleansing or respiratory functions that are continuously active. For example, while the control of lateral cilia of lamellibranch gills has been suggested several times, the frontal cilia of the gill filaments seem to be continuously active.

Many cases of ciliary control which appear to involve inhibition may in fact be cases of activation, and it is difficult to be certain which method of control is being used without thorough experimentation. The control of the lateral cilia of lamellibranch gills is a case in point. In *Mytilus*, Lucas (1931 a, b) found that the branchial nerves do not enter the gill filaments, so that the lateral cilia are unlikely to be under direct nervous control. These lateral cilia cease their activity shortly after the gill filaments are isolated from the body, and Gray (1926) found it necessary to modify ionic concentrations or add veratrine in order to keep the cilia beating. Aiello (1960) agreed that the lateral cilia of isolated gills cease beating about one hour after removal from the animal; he also found that if the branchial nerve on one side was cut close to the visceral ganglion, the lateral cilia of the gill on that side stopped after about an hour, while the cilia on the opposite gill continued to beat. He was led to conclude that the cilia were under control by activation, and, since serotonin increased the rate of beat and was normally present in the gill, he suggested that nerve activity caused the release of serotonin (probably as a neurohormone) into the gill tissue as an activator of ciliary movement.

On the contrary, Nelson (1951, 1960) has described the inhibitory control of lateral gill cilia of *Ostrea virginica*. The lateral cilia in early transparent spat of this species can be seen to cease their beating activity periodically for a few seconds or more, and then resume full activity again. This inhibition can be destroyed by cutting the filaments from the gill axis, or by application of chloretone. Nerves have been shown to run in the gill filaments of *Ostrea*, and the inhibitory influence is probably exerted by them.

The control by activation in *Mytilus* seems to be slow acting and less direct than the inhibitory control of *Ostrea*, yet it should be sufficient to reduce the activity of the cilia when not required, e.g. when the shell is closed for long periods. It is possible that control by activation is able to give a variation in the rate of beat according to the amount of activator liberated, while inhibitory control can only vary the activity by stopping the cilia for a longer or shorter period.

McDonald, Leisure and Lenneman (1927) believed that both activation and inhibition are used in the control of the rate of movement of frog pharynx cilia. They claim that stimulation of the sympathetic nerves increased the ciliary activity, while it was decreased by stimulation of the parasympathetic nerves. Although others, e.g. Seo (1931) and Lucas (1935) have also found the activation of these cilia by nerve stimulation, they have not found inhibition; in fact, Lucas found that the cilia are normally quiescent, but are activated by stimuli from nerves of parasympathetic origin. Lucas and Loren (1935) could find no change in the ciliary activity of turtle tracheal epithelium when the nerves supplying the trachea were stimulated.

A nemertine that moves by ciliary action was found by Friedrich (1933) to show a reversal response rather like that of *Paramecium*. If the head is touched while the animal is moving forwards, the cilia stop and then reverse to move the animal backwards. The cilia remain motionless after decapitation but, if the cut end is stimulated, the cilia will start to beat in reverse. It is difficult to determine whether this is a combination of inhibitory and excitatory control, or whether we ought to make an additional class for control which involves the reversal of ciliary beat, as suggested by Fedele (1926) after he had studied a number of metazoans.

Other important examples of inhibition of locomotory cilia should be mentioned. The planktonic veliger larvae of molluscs are moved by the fringe of large compound cilia on the velum. Carter (1926) found that these cilia do not beat continuously, but that they show periodic intermissions and may also stop if the animal is stimulated, so that it appears as if the ciliary beat is under the control of the animal. The cilia of isolated cells beat continuously without intermissions, and, in the presence of such narcotic drugs as nicotine or morphine, the intermissions of normal velar cilia ceased at concentrations which were too low to have any other obvious effect on the beating activity. The inhibition appears to be caused by the action of nerves which run around the velum from the cerebral ganglia and give off nerve fibrils whose endings are intracellular and near the basal bodies of the cilia (Carter, 1928). The ctenophore *Beroe* is moved by the action of comb-plates which Gothin (1929) found to be controlled by inhibitory impulses from the nervous system of the animal. Chloral hydrate (0·1 to 0·2 per cent) did not affect the automicity of the ciliary beat, but prevented inhibition. Inhibitory control from the nervous system is also said to control the locomotion of cilia of some of the smaller snails, e.g. *Alectrion* (Copeland, 1919), and the cilia of the lips of *Physa* (Merton, 1923).

In no case can we be certain of the means by which the control of cilia is exerted. The suggested activation mechanism of lateral cilia of *Mytilus* by serotonin is perhaps the nearest so far, although we do not know how serotonin may act on the ciliary mechanism. Electrical and membrane effects on the reversal of protozoan cilia may also be useful clues to the methods of control, but here again we have not yet got to the roots of the problem (see pp. 93 and 195).

13. Microdissection Studies on Ciliated Tissues

The technique of microdissection has been used in several ways in the study of ciliary activity. Most commonly it has been employed in experiments on the co-ordination of cilia, in order to find out if conduction was upset by cuts into the cells carrying the cilia. Similar experiments have been carried out to find out how much of the cell is necessary to maintain the beat of the cilia, and to determine whether the normal polarity of beat can be changed by re-orientation of the tissues carrying the cilia.

Verworn (1889) observed that a single cilium that had been isolated with a small piece of its basal cytoplasm was capable of continued beating, and Lepşi (1926) removed the cytoplasm of *Glaucoma* to leave an empty pellicle on which the cilia continued to beat for a short time. From these findings it appears that only part of the cell is necessary for beating activity.

Rather more refined studies were made by Worley (1941) on the epithelial cells of the intestine of some lamellibranch molluscs. He cut across the cells parallel to the ciliated surface at various levels in order to find the effect on beating and co-ordination. While cuts through, or immediately below, the basal bodies resulted in a complete stoppage of beating, cuts at deeper levels allowed the beating to continue, but prevented normal co-ordination unless the cuts were internal to the nucleus and ciliary roots.

Cuts through a row of cilia at right angles to the surface were found by Verworn (1889) to interrupt the metachronal co-ordination of the cilia in the row, e.g. the membranelles of *Stentor*, *Spirostomum* and *Euplotes*. Coonfield (1934) found a similar state of affairs in *Mnemiopsis*. More recently it has been shown (Sleigh, 1957) that a cut across the membranelle row of *Stentor* prevents the passage of the metachronal waves, and that new waves and a new rate of beat start in the region immediately distal to the cut. When the cut healed—in 15 min to an hour or so—the metachronal waves passed across the cut place as if a cut had never been made. Similar cuts across the ciliary rows of *Opalina* also obstruct the passage of metachronal waves, but the reversal response still occurs simultaneously on the two sides of the cut (Okajima, 1954a). The same results have been reported by Doroszewski (1958) in *Paramecium.* These observations confirm the findings of Worley (1934) in similar experiments on the body cilia of several ciliates. Taylor (1920) has investigated the effects of cutting the fibrils which connect the cirri and membranelles of *Euplotes* to the " motorium "; such cuts result in disorganization of the movements of these organelles, and it was therefore concluded that the fibrils function as conductors.

It seems fairly certain that the normal direction of ciliary beat, at right angles to the line through the two central fibrils, is determined in the formation of cilia, and that it remains unchanged throughout life, with the exception of periods of reversal where these occur. This conclusion comes from experiments in which areas of ciliated tissue have been cut out and grafted back into position with reversed polarity. Using this method, von Brüche (1916) found that cilia continue to beat in the same direction relative to their own basal protoplasm, and not relative to their new position. Lucas (1933) also found this to be true provided that the environment was unaltered. It is possible that the orientation of beat is not fixed immediately the cilia are formed, for Twitty (1928) found that while the cilia of the epithelium of very young amphibian embryos could change their beat direction to correspond with that of cilia of the surrounding region, the cilia of older embryos retained their polarity so that cilia of the graft beat in the opposite direction to those of the surrounding area.

This expression of polarity may be shown even more strongly by the grafting of some ciliated tracts in reversed orientation. Thus Tartar (1960) found that if sections of the membranelle band of *Stentor* are reversed, then proper reuniting of the section will only occur after it has turned itself back through 180°. The whole membranellar band then resumes a co-ordinated beat.

The same happens in the rows of comb-plates of *Mnemiopsis*, where Coonfield (1936) found that a reversed section would grow so as to link up the cut ends that had been severed; the original polarity of beat was retained throughout.

CHAPTER 4

THE MOVEMENT OF CILIA AND FLAGELLA

The structure of cilia is well known, and various parts are consistently present. It is proposed in this chapter to describe the form of beat of cilia and flagella, to attempt the allocation of functions to the component parts of these organelles, and to discuss the means by which these functions are carried out. The action of many factors on the activity of cilia has already been described, and knowledge gained from these studies will be incorporated into discussions here. Some of the ideas presented here are purely hypothetical, and it is hoped that the reader will be able to distinguish facts from speculation.

1. The Form of Beat of Flagella

It seems unlikely that the familiar pattern of flagellar beating in which the organelle takes the form of a sine wave is often found anywhere but in theoretical discussions. In all cases examined the pattern seems to depart from this ideal in at least some details. In fact, few flagella have been studied in sufficient detail for adequate description of their movement, with the exception of studies on the sperm tails of the sea urchin and bull by Gray (1955, 1958), in which the sea urchin was found to approach nearer to the sine wave pattern. Protozoan flagella normally show a modified beating pattern on account of their position and mode of functioning, and must be considered separately.

Gray (1955) established that the bending waves of the flagellar tail of the sea urchin sperm take place in a single plane. In sea urchin sperm tails that beat symmetrically, waves of bending arise at the head end of the tail and pass towards the tip, moving down opposite sides of the tail in strict alternation to give the pattern shown in Pl. XV a, b and c. The tail is somewhat longer than the length of one cycle of the sine wave bending pattern; if the tail were considerably longer, this form of beating would have the appearance of what has been regarded as the "typical flagellar beat".

When bending waves of this form are passed backwards along a flagellum, the water exerts forces against the tail in such a way that the whole body is moved forwards. The forces acting on various parts of the body will differ, and at any instant they will have both transverse and longitudinal components for all elements of the tail that are moving (i.e. excepting those at the crests of the waves). The maximum longitudinal forces will be exerted on those elements of the tail which are moving at a maximum

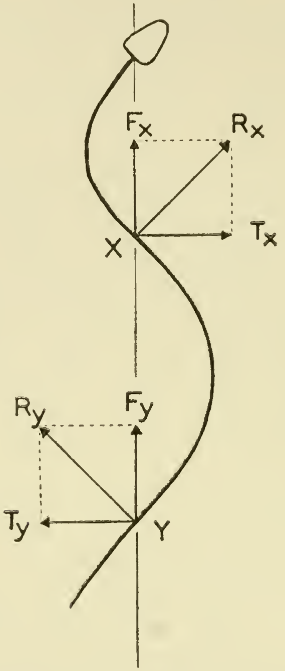


FIG. 30. Simplified diagram of forces exerted by the water on two elements of a sperm tail of a sea urchin. The elements *X* and *Y* are being moved transversely because the bending waves in which they occur are moving backwards. Thus, element *X* is moving to the left and element *Y* to the right. The forward components *Fx* and *Fy* act together, but the transverse components *Tx* and *Ty* are in opposition. (The forward movement of the sperm is neglected.)

angle to the axis of progression, e.g. at points **X** and **Y** in Fig. 30. It will be seen from this simplified diagram that the longitudinal forces on all elements of the tail tend to move the body forwards, while transverse forces oppose one another, and, provided the tail is as long as or longer than the length required to form one complete wave, the resultant transverse force will be somewhere near zero and will balance approximately about the mid-point of the tail. The longitudinal thrust on the body tending to produce forward movement is opposed by a backward drag which is mainly due to viscous forces, for at this order of magnitude and in this type of medium, inertia of the body has a negligible effect in comparison with the viscous resistance of the medium (Bidder, 1923; Taylor, 1951).

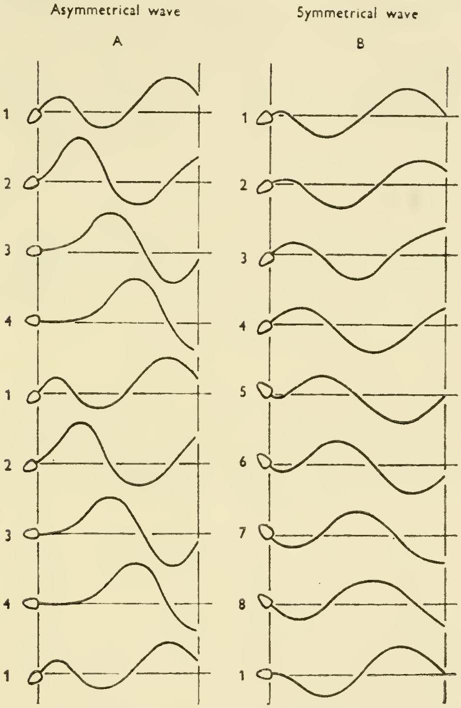


FIG. 31. The movement of sea urchin sperm tails. **A,** Two cycles of bending of a sperm tail with an asymmetrical beat, and **B,** one cycle of bending of a sperm tail with a symmetrical beat (from Gray, 1955).

The movements of the sea urchin sperm have been shown by Gray and Hancock (1955) to approach closely to the ideal theoretical pattern. They derived an equation expressing the rate of forward movement of sea urchin sperm in terms of the amplitude and wavelength of the bending waves, the number of waves in the tail length, the frequency of waves, the radius of the tail and the radius of the head. Substitution of measured values of these parameters in the equation gave a calculated speed of movement of 191 μ/sec, while the average observed speed was 191·4 μ/sec.

The simplicity of the pure sine wave pattern, even in sea urchin tails with a symmetrical beat, is usually complicated by an increase in both wavelength and amplitude as the bend passes down the tail. The beat is further complicated in the majority of sperm tails by an inequality of bending on the two sides, so that the wave pattern is asymmetrical, one side showing a larger amplitude and longer half-wave than the other as in Fig. 31. Such an asymmetry of bending will have important effects on the movement of the sperm.

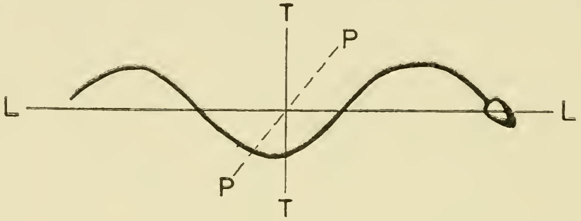


FIG. 32. Diagram of a sperm to show the three axes mentioned in the text. The oscillations of the tail take place in plane *T–T*; the axis *P–P* is at right angles to both *T–T* and the longitudinal axis *L–L*.

Three axes of the sperm may be defined as in Fig. 32 by *L-L*, *T-T* and *P-P*, the last being perpendicular to both the transverse and longitudinal axes of the sperm (the waves of bending are here assumed to be in the transverse plane). Movements about these three axes are designated as follows: movements about *L-L* constitute a roll, about *T-T* a pitch, and about *P-P* a yaw. Where bending of the sperm tail is asymmetrical, the sperm will persistently yaw towards one side or the other because the transverse forces acting on the tail will not balance about its mid-point, and consequently the sperm will tend to move in a circle. In addition, however, all sea urchin sperm seem to roll, and a combination of yawing and rolling results in a spiral movement of the sperm about a straight line, which is the axis of progression. The greater the yaw, the larger the diameter of the spiral, and the faster the roll, the smaller the diameter of the spiral, if the rate of forward movement is constant. Pitching movements seem to occur rarely and are probably of lesser importance.

The presence of a yaw component in the movement of a sperm is easily accounted for by an asymmetry of bending, but a rolling component requires more explanation. It appears that the majority of sea urchin sperm roll in an anticlockwise direction (looking along the axis of progression), and earlier observers attributed this to an asymmetry of the head, but both Gray (1958) and Bishop (1958b) believe that rolling of sperm (of bull and squid respectively) takes place because the beating of the terminal part of the tail is not in quite the same plane as that of the main part of the tail. A slight twist in the plane of beating of the tail of a moving sperm could easily cause such a roll, but Bishop found that the rolling movement occurred in stationary squid sperm that were motionless except for oscillations of the distal end of the tail. A sea urchin sperm whose tail is beating with a frequency of 30 or 40 c/s may roll with a frequency of 0·5 to 3·0 per sec, so that a relatively small motion of the tail may be sufficient for rolling.

It seems well established, both from observations on sea urchin sperm by Gray (1955) and the calculations of Machin (1958), that the bending movements of sperm tails are not the result of waves propagated along a passive structure from a driving system at the attached end, but that active contractile elements are situated along the length of the flagellum and energy for bending is contributed throughout this length. The most probable site for contraction is the ring of nine peripheral fibrils; bending of the flagellum could be caused by a localized shortening of some of these fibrils (see p. 146). The asymmetrical waves of bending evidently result from unequal contractions on the two sides of the tail, and this in turn may result from an asymmetry of the timing of contractions on the two sides.

The movement of bull sperm is further modified from the sine wave pattern; in fact, Gray (1958) suggested that while the sperm of the sea urchin move like eels or snakes, the movements of bull sperm resemble those of fish like the trout. This appears to result from the fact that the anterior part of the bull sperm acts as a fulcrum against which the posterior part of the tail can exert a propulsive force; in the sea urchin sperm all parts of the tail show similar lateral movements. The difference between the movement of these two types can no doubt be correlated with the difference between the structure of the two sperm, for the more complex anterior region of the anterior part of the mammal sperm tail may reduce the flexibility of this part of the tail. The bull sperm also differs from the sea urchin sperm in that the head is relatively larger.

A moving bull sperm (Pl. XV f-m) shows waves of bending which resemble those of the sea urchin in that the amplitude of the bending wave progressively increases towards the distal end, while the tail is long enough to accommodate rather more than one complete wave. They differ in that bending waves of the bull sperm show a decreased speed of propagation of the waves and a decreased wavelength as the waves approach the distal end. The proximal part of the tail bends little and the distal part moves much more in broad sweeping movements, so that the " optical envelope " traced out by the bull sperm in motion is roughly triangular (Pl. XVe), while that of the sea urchin is more nearly elliptical (Pl. XVd). The distal tip of the tail of the bull sperm traces out figure-of-eight movements, so that during part of the beating cycle the tip may even be moving forward relative to the head. These movements are comparable with those of the tail of a trout-like fish.

During a large part of the transverse movement of the distal region of the tail, it is inclined at a large angle to the axis of progression, and so is able to exert a large propulsive force compared with that of a more proximal region of the tail with a smaller angle of inclination (see Fig. 33). Transverse forces are relatively larger where there is a small angle of inclination, but, provided there is more than one wavelength in the complete tail, the resultant force transverse to the axis of progression will be near zero. It seems that the relative inflexibility of the anterior

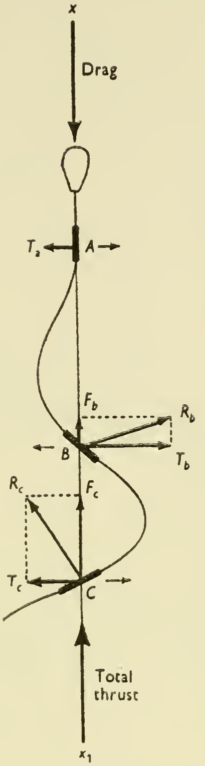


FIG. 33. Diagram of the forces exerted by the water on three elements of the tail of a bull sperm. The waves of bending are moving posteriorly and elements *A* and *C* are moving to the right while element *B* is moving to the left (as indicated by small arrows). The reactions to the movements of these three elements are shown by *Ta*, *Rb* and *Rc*. The components *Fb* and *Fc* act together in moving the sperm forwards, and are opposed by drag acting in the whole sperm, while the transverse components *Ta*, *Tb* and *Tc* more or less cancel out. Compare this with Fig. 30 and note that here the increased angle of inclination of element *C* gives an increased forward component *Fc* (from Gray, 1958).

region permits it to be used as a fulcrum against which the tail can push, with the result that a transverse force generated there can balance forces due to distal bending waves.

Pronounced rotational movements are found in bull sperm, but the occurrence of yawing movements seems less frequent than in sea urchin sperm. The absence of yaw may be only apparent, for the rotational movements are rapid, and would tend to obscure the tendency to yaw; indeed this may be the purpose of rotation. It is almost certain that the rotational movements take the form of a rolling rather than a rocking motion, and that although the main part of the tail traces out a lamina in a single plane, the distal part of this lamina may be twisted. It is likely that the twist here is more pronounced than in sea urchin sperm because the frequency of rotation is nearer to the frequency of bending waves. As the head of the sperm rocks and rolls under dark ground illumination, its flattened surface reflects light in flashes; Gray found that the average frequency of flashing was 8·2 per sec, while the average frequency of bending waves was 9·1 per sec. The twisted beat of the terminal part of the tail may be connected with the fact that the ordered arrangement of fibrils is lost in this region (see p. 43).

Rather similar bending waves propagated along a flagellum may thus be used in different ways to produce forward progression of these two types of sperm. The reasons for the difference may be that: (1) the flat head of the bull sperm is nearly 3 times as long and 4 or 5 times as wide as that of the sea urchin sperm, and will increase the drag opposing forward movement; (2) the sperm of mammals may be required to swim for a longer time than those of the sea urchin, hence the larger mitochondrial sheath and the thick mid-piece of the bull sperm.

The movement of mono- and bi-flagellate protozoa has been studied by Lowndes (1936-45) and Brown (1945). It appears from this work that in almost all cases, as well as in mono- and bi-flagellate zoospores and sperm of plants, the flagella are used in a quite different way from that described for animal sperm tails, probably because the "head" structure is very large compared with the "tail" thickness in the protozoan. The flagellum or flagella emerge from that end of the animal which is anterior when it is moving, and their beating causes a rotation and gyration of this end of the animal, such that the animal " screws " its way through the water by the principle of the rotation of an inclined plane (Fig. 34). The mode of movement of the organism need not be considered at length here, but the motion of the flagellum is of interest.

Lowndes (1944) has pointed out that the description of flagellar movement of *Monas* given by Krijgsman (1925) in his figures A and B, and since copied many times, does not give a good idea of the normal movement of the flagellum of this organism. In the first place, Krijgsman stated that his figures were diagrammatic or semi-diagrammatic, which Lowndes interpreted to mean that they were Krijgsman's impressions of the way in which the flagellum moves. Secondly, the movement of *Monas* is inhibited by strong light, in which the organism executes only rather feeble " kicking movements ", so that, under the illumination necessary for normal high magnification investigation of movement, *Monas* showed a maximum swimming speed of about 10 μ/sec. In feebler illumination it was found by Lowndes to swim at about 260 μ/sec, so that it is unlikely that Krijgsman was in fact seeing *Monas* moving forward at maximum speed. In any case, one could not watch the flagellum of an animal that was moving at that speed under the high magnification necessary to see it. It is rather sad that these diagrams, which have been used so often to convey an idea of the movement of a flagellum, should be found to show an abnormal mode of movement, but they have had their value in showing movements that flagella can perform, and indeed they show a cycle of movement not unlike that of many cilia.

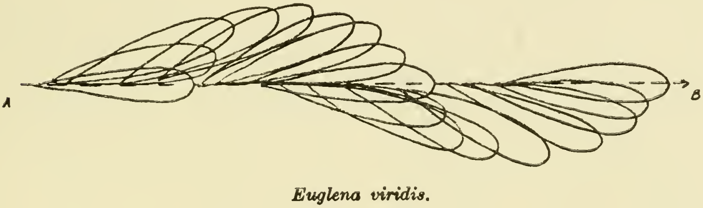
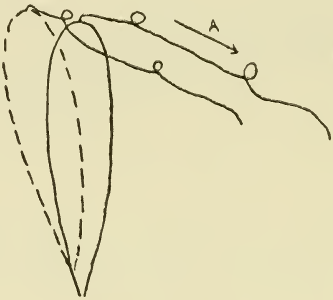


FIG. 34. Movement of *Euglena*, showing the successive positions taken up by the organism in swimming along the line *A–B* (from Lowndes, 1941).

It is a pity that Lowndes was not able to substitute for these diagrams an equally clear picture of the movement of a flagellum from his own observations. He can not be blamed for this, however, for the movements are evidently not simple, and two features in particular complicate the picture very considerably. The first is that the movements are three-dimensional in that the body of the organism rotates even though the flagellar beat may be more or less in a single plane. Secondly, the flagellum projects from the anterior end of the body as the organism moves, and the movement of the water is bound to bend the flagellum back and distort the movement of bending waves propagated from the base of the flagellum to the tip.



Euglena viridis.

FIG. 35. Movement of *Euglena*, showing two waves of contraction passing along the flagellum in the direction indicated by the arrow. The movement of these contraction waves tends to move the organism towards the dotted figure (from Lowndes, 1941).

In all cases Lowndes found that the flagellum is moved by waves of bending that pass from the base of the flagellum to the tip, there being sometimes one and sometimes several waves in the length of the flagellum, depending on the length of the flagellum and on the rate of propagation of the bending wave. The flagellum is bent back to lie roughly parallel to the body, and waves of bending propagated along the flagellum were found by Lowndes to cause a rotation of the flagellar tip, because the long distal part of the flagellum was not easily moved in the water, and the most stable position was that in which the contracted region of the flagellum was twisted into a loop (Fig. 35). The result of the passage of these bending waves is to spin the organism on its axis, and to cause the anterior end of the animal to gyrate in such a way as to cause forward motion. These findings have been confirmed by the observations of Brown (1945).

The movement of the anterior flagellum of *Peranema* deserves special mention because it has often been misunderstood; in spite of the fact that Verworn (1899) and Lowndes (1936) have described it correctly, many authors have given incorrect accounts. In the normal forward " gliding " movement, the long anterior flagellum is held straight out in front, and undulations are found only at the tip, passing in the direction from base to tip. The anterior flagellum is unlikely to cause this gliding component, whose mechanism is still not understood, although we now know that there is a second flagellum passing along the body surface. The second type of activity shown by *Peranema* is a writhing movement with active lashing of the anterior flagellum, which results only in a circling movement with little or no forward component, and may result from stimulation of the animal. These flagellar contractions are more like those of typical flagellates and show two interesting features. Lowndes (1941) found that the velocity of propagation of the contraction wave increases along the flagellum from base to tip, and the amplitude of the waves also increases. The change in form of the bending wave as it passes along this flagellum may be connected with its complex structure (p. 39), for the tapered intraflagellar strand could give a decreased resistance to bending towards the tip, and the striated sheath may also modify the movement.

Patterns of beating from a more or less pure sine wave to a unilateral ciliary beat are found among the more complex flagellates. Two examples from the gut of the termite *Zootermopsis* will serve to illustrate this. *Trichomonas termidopsis* has four free flagella and a fifth, recurrent flagellum forms part of the undulating membrane. In life, the undulating membrane almost encircles the body and waves of contraction pass along the recurrent flagellum from base to tip in continuous succession, causing the body to rotate. The bending waves of the recurrent flagellum appear sinusoidal, with the plane of bending roughly at right angles to the plane of the undulating membrane, although it frequently looks twisted, so that this observation needs confirmation.

The anterior flagella of this trichomonad (at least in preparations of animals removed from the termite and examined in a saline solution) perform a cycle of beat rather like that of many cilia. The four flagella frequently beat synchronously, but may move separately through the characteristic cycle. At the beginning of a beating cycle the flagellum is held straight out, and bends at the base through an angle of about 90° while the main (distal) part of the shaft remains straight. A wave of bending then passes up the flagellum from the base bringing the flagellum back to the starting position. This cycle of movement is illustrated in Pl. XVIa.

Several species of large flagellates of the genus *Trichonympha* are also found in this termite. The flagella of the body of *Trichonympha* occur in enormous numbers and are inserted on the body in such a way that they are directed backwards. Undulations of these flagella appear to be approximately sinusoidal, and bending waves passing backwards along many flagella at the same time are sufficient to move this relatively large organism (see Pl. XVIb). Pitelka and Schooley (1958) have pointed out that this flagellar beating will only move the animal forwards, and that changes of direction are caused by movements of the mobile anterior end (rostrum) of the body.

2. The Form of Beat of Cilia

The difference between the form of beat of flagella and that of cilia has usually been regarded as a fairly large one, but this is most probably a mistake, for the ciliary beat appears to be but a modification of the flagellar beat. Gray (1955) found that the beat of the sea urchin sperm tail was often asymmetrical. A sperm tail with extreme asymmetry shows a beating pattern very similar to that of a cilium. This is illustrated in Fig. 36, where, although the positions of the flagella in parts A, B and C are falsified because the head normally moves from side to side, the transition from the symmetrical flagellar beat to the ciliary beat is clearly shown.

Few reliable observations of the beat of cilia have been made, but they usually agree in that the beat of a cilium takes place

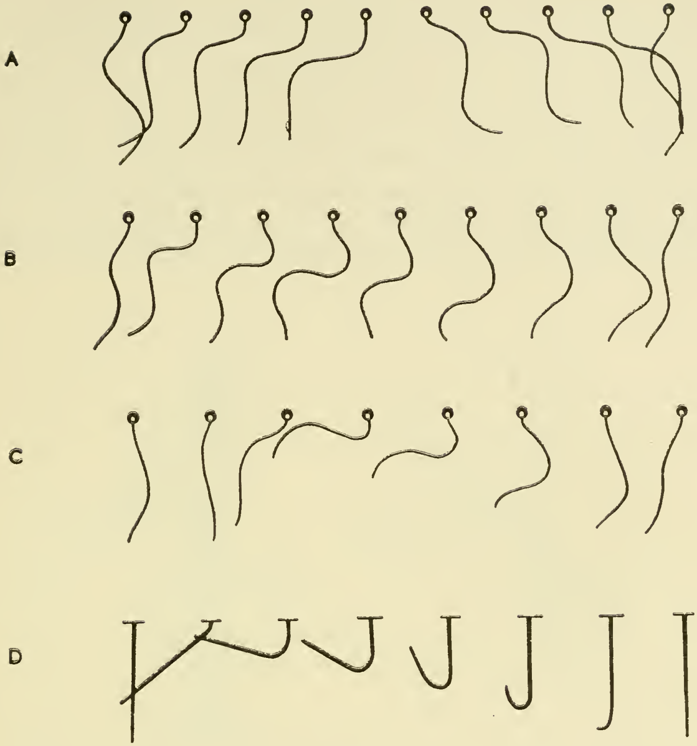


FIG. 36 Diagram illustrating the supposed transition from a symmetrical flagellar beat to an asymmetrical ciliary beat. The outlines in **A**, **B** and **C** show the successive shapes and positions of sea urchin sperm tails relative to their heads, which are drawn as if they were fixed; in the movement of these sperm the heads of course moved from side to side. **A** represents a cycle of beat of a symmetrically beating sperm tail, in **B** the cycle is moderately asymmetrical and in **C** it is very asymmetrical. **D** represents a cycle of beat of a membranelle of *Stentor*. (Sea urchin sperm outlines drawn from Gray, 1955; **A** and **B** were taken from outlines shown in Fig. 31.)

in a single plane, and that a bending wave is propagated up the cilium from base to tip. In some very interesting work using a rapid fixation technique, Párducz (1954) found that the ciliary beat of the body cilia of *Paramecium* does not take place in a single plane, for in the recovery stroke the cilium swings out to the side, so that the tip of the cilium follows an almost circular path instead of moving to and fro in the same plane. Although it is unwise to lay too much stress on the analysis of the shapes and positions of cilia of fixed animals, especially those in which the direction of beat is known to change practically instantaneously in response to a variety of stimuli, the cilia of *Paramecium* have been shown by Párducz to be worthy of an intensive study in the living state. Some observations on the beating of vestibular cilia of *Paramecium* by the author did not reveal any obvious movement of the cilia out of a single plane.

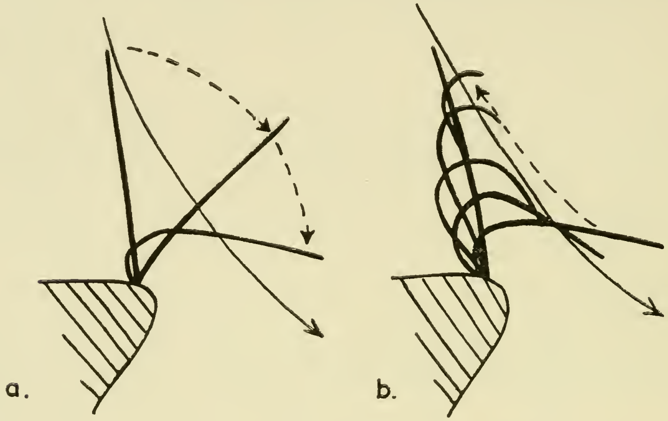


Fig. 37. Movements of a membranelle of *Stentor* in relation to the current of water that they cause. The dotted arrow indicates the movement of the cilium and the full arrow indicates the movement of the water. (**a**) The effective stroke, and (**b**) the recovery stroke. (See text.)

Because of the mode of use of a cilium, its effective activity is unidirectional, so that the beating cycle of a cilium can be separated into an effective phase in which most of the useful work of the cycle is performed, and a recovery phase in which the cilium returns to the position required to commence a new effective phase. For maximum efficiency of the effective stroke, the cilium should present as large a surface as possible perpendicular to, and yet moving parallel with, the required direction of movement of the medium. In the recovery phase the cilium should present the smallest possible surface to the moving medium, and is therefore trailed through the water (see Fig. 37).

The beating of the membranelles of *Stentor* is easily observed under stroboscopic illumination, especially at the edge of the peristome where the cilia appear as in Fig. 37. The sequence of movements of these cilia in one complete cycle is shown in Fig. 38. The effective phase starts with stage 1, and has nearly ended by stage 3. The wave of flexure which causes the recovery phase has started to move up the cilium before the effective phase has been completed, and, by the time it has reached the tip of the cilium, the cycle of beat is complete. It is interesting that the effective and recovery phases are not separate parts of the beating cycle, but that they overlap, merging together in a single movement that could be caused by a single wave of contraction passing up the cilium from the base.

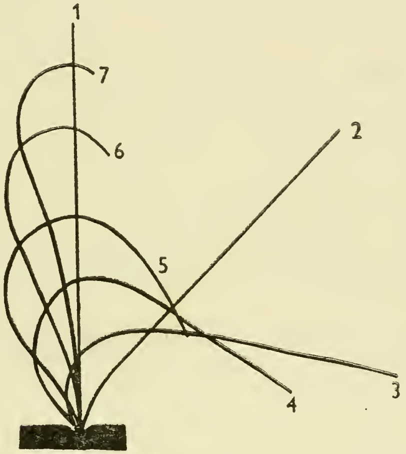


FIG. 38. The sequence of movements of a membranelle of *Stentor* (from Sleigh, 1960).

The movements of the cilia of *Opalina*, on the other hand, are more like those of flagella in many respects (Figs. 39 and 47, and Pl. XVIIIb); in fact, it might be more satisfactory to regard them as flagella in agreement with the findings of Grassé (1952) and Corliss (1955), who maintain that *Opalina* shows more affinity with flagellated than with ciliated protozoa. These cilia appear to be rather flexible organelles, and the long ones at the posterior end of the body trail in the water in a manner reminiscent of the flagella of *Trichonympha*. It seems best to regard the " resting position " of these cilia as that in which they lie close to the body surface (Fig. 39, stage 1). A wave of bending passes up the cilium, straightening it out in a preparatory phase equivalent to the recovery phase of the *Stentor* ciliary beat. Before this wave of flexure has reached the tip of the cilium, a second bending wave on the other side of the cilium pulls the shaft back towards the body surface in the effective phase. The cycle of beat is completed when the second bending wave has been propagated to the end of the cilium. In the preparatory phase the cilia are lifted from the body surface in a smooth curve (see Fig. 47, p. 176) and in the effective phase (i.e. that in the direction of the effective movement of water) they are brought back to the body surface. It seems likely that movements of the complete waves are responsible for the movement of the organism through the water, rather than movements of individual cilia; in this also the motion of *Opalina* resembles that of *Trichonympha* (see also

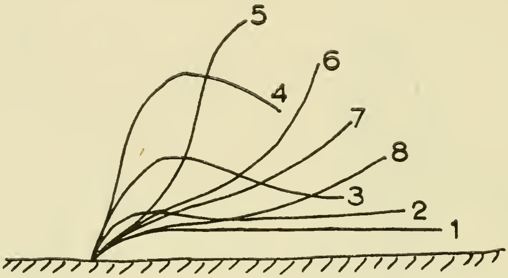


FIG. 39. The sequence of movements of a cilium of *Opalina*. Compare this with Fig. 47 and Plate XVIIIb, where these cilia form part of metachronal waves.

p. 175). The form of beat of *Opalina* cilia appears to be the same in both normal and reversed swimming, although the direction of beat is changed.

Abfrontal cilia of the gills of *Mytilus* have been found to be ideal for the study of the form of ciliary beating. Gray (1930) published an excellent sequence of cine-micrographs showing the cycle of beat of these cilia, and several workers have used them for physiological studies on ciliary movement (e.g. Kinosita and Kamada, 1939; Yoneda, 1960). The cilia at rest lie along the gill surface, and during each cycle of beat the cilium first straightens out, by the passage of a wave of flexure from the base to the tip, and then swings back to the gill surface as an apparently stiff structure (Pl. XVIIa).

Unfortunately this pattern of beat is unusual if not unique. It is difficult to tell which part of the beat is the effective stroke in the sense that it is responsible for the main movement of water. The effective stroke of most cilia occupies a small fraction of the total beat, say $\frac{1}{4}$ to $\frac{1}{3}$ at most, and during this stroke the cilium moves stiffly through the water as a result of a bend at the base. The second part of the beat of *Mytilus* abfrontal cilia is equivalent to the effective stroke of many cilia in that it is the part of the beat in which the cilium is moved stiffly through the water, but it occupies something like $\frac{7}{8}$ of the total cycle (Kinosita and Kamada, 1939). For much of this part of the beat the cilium moves very slowly through the water. In the first (preparatory) phase of the beat the cilium straightens out very rapidly, appearing to " flick " the water. Most of the effective water movement must be done during this phase, and perhaps this reflects the function of these cilia, which is probably to prevent the settlement of small organisms (protozoa, etc.) on the abfrontal surface of the gill. These cilia are also unusual in that they occur singly among many short cilia; all abfrontal cilia on any one gill filament do not necessarily beat with the same orientation.

The appearance of the beating of these cilia suggests that the most satisfactory explanation of the cycle of beat in this case is that only the preparatory phase is active, while the " effective " phase is passive; in most cilia both phases are believed to be active (see pp. 153 ff.). Thus, if the natural shape of the cilium were to hold it close to the gill surface, and a wave of flexure passing up the cilium were to work against this and straighten out the cilium, then the elasticity of the cilium might be responsible for the return of the cilium to the resting position. It is interesting in this respect that an abfrontal cilium in a hypertonic medium (3g NaCl in 100 ml seawater) showed normal amplitude and normal duration of the preparatory stroke, but the duration of the " effective " stroke was nearly twice the normal value (Kinosita and Kamada, 1939). In addition, the resting periods, which occur from time to time, always occur between effective and recovery phases, i.e. when the cilium is in the contracted position according to the usual theories. Most cilia rest in the relaxed position if they rest at all.

The calculations of Harris (1961) (quoted at length on pp. 146 ff.) have a bearing on the interpretation of the beating of these cilia. Harris deduced in this work that, for a single cilium, the time taken for the effective stroke should be proportional to the cube of the cilium length, while the time for the recovery stroke is related to the time taken for the wave of flexure to pass up the length of the cilium, and is thus proportional to the cilium length. In a long cilium, therefore, the effective phase may occupy a much larger fraction of the whole beating cycle than in a short cilium. These abfrontal cilia are compound, which modifies this picture since more energy is available for moving the cilium against water resistance; the resistance to bending is assumed to be always proportional to the bending force, i.e. the same ratio for a compound cilium as for a single one. These arguments may be part of the answer, but cannot provide the whole answer, for abfrontal cilia showing the same form of beat may vary in length from some 20μ to 100μ, and other cilia within this size range may occur on the gill and show a quite different form of beat (compare series (a) with series (b) in Pl. XVII).

From this extensive discussion it must be obvious that the beat of these cilia can not be explained by any conventional theory, and it would be wise if further experimental work, as well as an electron microscopic study of structure, were to precede any further speculation.

Some of the long cilia that occur among the frontal cilia on *Mytilus* gill filaments show a form of beat that appears identical with that of the normal frontal cilia, and is easier to see. The beating of such a cilium is shown in Pl. XVIIb. This pattern of beat was described and figured by Gray (1922a) from observations on cilia from the same source. A very similar form of beat is shown by cilia on the branchial lobes of the tubicolous polycheate *Sabellaria* (Fig. 40a), and the beat of the vestibular cilia of *Paramecium* appears basically the same (Fig. 40b), although the amplitude of beat may be a little larger. The slight difference in the appearance of the beat may be related to the metachronism of the cilia, for that of the cilia of *Sabellaria* is diaplectic, while that of these cilia of *Paramecium* is antiplectic (see p. 174); the latter cilia may interfere with each other in the recovery stroke.

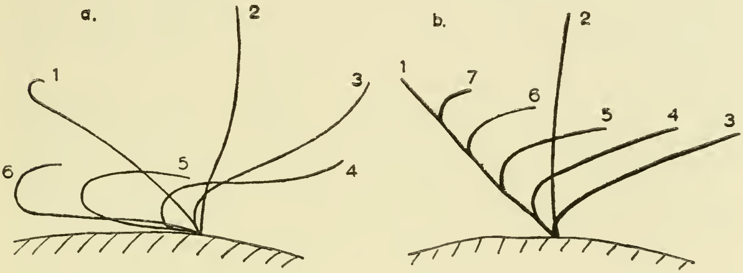


FIG. 40. Diagrams showing the sequence of movements of cilia of (a) *Sabellaria* and (b) *Paramecium*.

Notice particularly that the recovery stroke starts before the effective stroke is complete.

Cilia do not all show the same form of beat, and the beating of many more types of cilia must be accurately described before we can attempt any classification of beating patterns. No " pendular " movements of the type figured by Gray (1928) have been described from cinematograph studies, and the evidence points to the fact that all ciliary beating cycles come under Gray's heading of " flexural " movements.

3. The Functions of the Parts of Cilia

The structure of cilia is well-known and consistent, but there is little evidence as to the functions of the various parts. A motile cilium must contain both contraction and compression elements, and certain structures seem well placed for the performance of these functions. The propagation of the wave of contraction along the cilium must also be accounted for in any theory of the mechanism of beat.

The nine peripheral fibril doublets are placed in the ideal position to act as the contractile structures of the cilium, since they could bend the cilium simply by shortening. The peripheral position of these fibrils permits some of them to exert a reasonably large bending moment about an axis through the centre of the ciliary shaft, and their distribution is such that bending moments caused by equal contractions in all fibrils on the two sides of any axis through the centre of the shaft balance very closely. Nelson (1958) has found that ATP-ase activity seems to be localised in these peripheral fibrils of the cilium, so that the energy supply is available there.

What part of the cilium then can function as the compression element to maintain ciliary rigidity ? The two central fibrils are absent from many non-motile cilia modified for sensory functions (p. 32), so that it appears as if they may have a mechanical function. Harris (1961) has recently calculated the bending couple necessary to overcome the resistance of the medium in the effective stroke of a cilium. On the basis that the rigidity of the cilium must be able to resist this bending couple, he found that the central fibrils would require a Young's modulus approximating to that of steel wire if they were the only compression elements, and they would therefore need to be much stronger than any known biological material. As an alternative source of rigidity Harris suggested that internal turgor pressure acting against the elastic tension of the ciliary membrane might provide sufficient stiffness, since no internal structures were likely to be strong enough. He proceeded to show by calculation that both the turgor pressure required and the Young's modulus of the membrane structure necessary to resist the bending couple were within reasonable limits for this sort of structure.

In these calculations Harris assumed that the bending moment of the effective stroke would be equivalent to half the couple exerted in the rotation of an ellipsoid twice the length of the cilium. With some minor approximations the bending couple can be expressed by $C = \frac{2}{9}\pi\eta l^3\omega$ (where C is the couple, η is the viscosity, l is the cilium length and ω is the angular velocity). It will be seen that this is the couple required to overcome the external resistance to movement, and takes no account of internal resistance.

Substitution of known values for *Paramecium* cilia in this equation gave Harris a value for C of 5×10^{-10} dyn cm. It is also interesting to compare the value found by substitution of figures measured for *Stentor* membranelles, which we have seen to be compound cilia built from 60 to 75 simple cilia. Such substitution gives a value for C of 3×10^{-8} dyn cm. Since Harris suggested that the bending couple of a compound cilium composed of n simple cilia should be nC, the couple due to each component cilium of the *Stentor* membranelle works out at 5×10^{-10} dyn cm or a little under—a remarkable agreement for a much larger structure with a faster beat.

Further calculation led Harris to conclude that if a bending couple of 5×10^{-10} dyn cm is required to bend a length of the cilium into an arc of radius 6μ, and if the central fibrils were responsible for maintaining stiffness, they would have to have a Young's modulus of 3×10^{11} dyn/cm^2 (that of biological fibrils is unlikely to exceed about 10^9 dyn/cm^2). If, on the other hand, it is assumed that turgor pressure is acting against the ciliary membrane to maintain the required rigidity, the Young's modulus for the membrane need be only 5×10^8 dyn/cm^2, and the internal turgor pressure required would be about $7{\cdot}6 \times 10^{-7}$ dyn, which could be provided by the osmotic pressure due to a concentration difference across the membrane of about 0·03 mol (assuming a non-dissociating molecule); all of these last three values are thought reasonable.

Additional evidence may be used to support the idea that internal pressure maintains rigidity. For example, Bradfield (1955) mentioned that the ciliary membrane nearly always appears wrinkled in fixed and dehydrated specimens whether the fixitive is hypotonic or hypertonic, and it is interesting that a large increase or a large decrease in the external osmotic pressure will stop the activity of lamellibranch gill cilia (p. 94). Also, where additional contractile material is present, as in mammalian sperm, the shaft is strengthened by a thickening of the outer layer of the ciliary cylinder; this could maintain the stiffness of the shaft both by increasing the tension the ciliary membrane can exert, and by acting as a spring by virtue of its spiral construction. The sheath of the sperm tail is especially well developed in the bandicoot sperm where the outer contractile fibrils are very thick.

Evidence which may count against this idea comes from the undulating membrane of some flagellates (p. 38), where the axial bundle of fibrils lies near one side of a greatly expanded flagellar membrane. In these cases, however, some additional material is usually present, and this may resist compression within a modified flagellar organelle; some people regard this additional material as being contractile, and its position does suggest this.

As an alternative hypothesis, it is possible that rigidity is provided by all of the fibrils of the axial bundle itself. If these have a spiral or tubular sub-structure, then they may be resilient and elastic, except during contraction when they may be under the influence of a plasticiser (e.g. ATP) and capable of shortening and extension.

Carter (1924) found that the effective stroke of *Mytilus* latero-frontal cilia could be arrested by a needle, while in the recovery stroke the cilium slipped past the needle. This led to the idea that cilia were stiff in the effective stroke and limp in the recovery stroke. However, Harris (1961) has pointed out that Carter's results could also be explained if it was assumed that the cilium was stiff throughout the cycle. This, on the whole, seems the more reasonable hypothesis, and no good evidence is available to refute it.

Several authors, e.g. Bradfield (1955) have suggested that the two central fibrils may be responsible for the conduction of the stimulus for the contraction of the peripheral fibrils, and yet others have suggested that the membrane may be responsible for this conduction. While either of these ideas could be correct, it seems that the nine contractile fibrils themselves are the most likely candidates. In many sensory cilia the two central fibrils are missing, and yet the cilia are still capable of carrying information, probably by the means normally used to conduct the contraction stimulus (and involving fibrillar contraction ?). The sensory cilia possess an outer membrane, and, in view of the fact that the membrane is the important conducting structure in nerve, it might hvae the same function here. In many sensory cilia the conducted impulse seems to arise in the peripheral fibrils or in structures continuous with them, e.g. in the retinal rod the flattened sacs of the photoreceptor region appear to be in continuity with the peripheral fibrils of the connecting cilium, while in cilia which detect mechanical vibrations distortion of the peripheral fibrils may initiate the sensory impulse (see pp. 32 ff.).

What function can then be allocated to the two central fibrils ? We know that in most motile cilia the two central fibrils are orientated in such a way that the line through their centres is at right angles to the plane of movement, and that many workers have found cross-links between the central and peripheral fibrils, so that it looks as if the central fibrils may have a mechanical function. If one imagines a cylinder whose rigidity is maintained by internal turgor pressure, and which contains a contractile structure running from end to end without any intermediate attachments, then the shortening of the contractile structure would result in the cylinder being transformed into a spiral, very much like the contractile stalk of a peritrich ciliate. In haptonemata, where central fibrils and cross-connexions have not been identified, the contraction appears to take this form, but in cilia and flagella it does not. It seems reasonable, then, to suggest that the central fibrils and the radial strands between the central and peripheral fibrils are responsible for localizing the effects of contractions to the particular region of the cilium in which they are taking place. Perhaps the arms and other links also play a part here, and it is interesting that at the tips of the cilia the orderly arrangement of peripheral fibrils disappears at the same time as the arms and radial links. Where an additional ring of supposedly contractile fibres is present in sperm tails, there is always some connexion between the outer and inner rings of fibrils; these could again function to localize the effects of contractions.

Perhaps it would be appropriate at this point to consider the possible reasons why there are 9 outer and two central fibrils. Astbury, Beighton and Weibull (1955) suggested that the arrangement of the 9-plus-2 fibrils of similar size is such that if there are two central fibrils, then 9 is the smallest number of outer fibrils which can form a ring around them and yet allow the inner pair to spiral relative to the outer fibrils. This is probably not a good enough reason, since the fibril structure is much more complex than these authors believed, and the inner fibrils are not known to spiral. Other suggestions have followed rather similar lines, but with the increase of our knowledge of ciliary structure and function, it should be possible to put forward some more valid reason.

It is interesting that centrioles, even in non-ciliated cells, usually possess 9 groups of longitudinal fibrils; it is by outgrowth of these 9 structures into the ciliary bud that the 9 peripheral doublets of the cilium are formed. It is probably not reasonable to assume that all centrioles originated from ciliary basal bodies, so that the number 9 may have its origin in the basic pattern of centriole organiztion rather than in ciliary organization. The answer to this part of the problem may therefore have to be sought elsewhere than in the cilium. It is certainly a most intriguing problem, and may be concerned with requirements of intermolecular and interfibrillar separation, along similar lines to the suggestions of Serra (1960), although these ideas themselves do not take sufficient account of the known structure of the cilium.

Haptonemata should fit into this picture somewhere, but, until information is available on their basal structure, any suggestions can be no more than speculation. For example, do the 6, 7 or 8 fibrils of the haptonemata that have been described represent a reduction from an original structure with nine contractile fibrils which developed from a typical centriole ? It is interesting to speculate further on the possibility that the haptonema was ancestral to the flagellum, and that an undirected movement and unlocalized contrcation were organized by the addition of a central structure which allowed cross-connexions to localize the contractions. It seems that the presence of two central fibrils is connected in some way with the fact that cilia and flagella normally beat in one plane, and it is interesting that the cross-connexions between the fibrils connect them more or less in two groups, each with four outer fibrils (i.e. 2, 3, 4 and 5 and 6, 7, 8 and 9) and one central fibril and sharing one outer fibril. The beat may be confined to one plane by balanced contractions in these two groups, which are symmetrical about the axis (*W*-*V* in Fig. 41b) of the flagellum or cilium that is in the plane of beat. In this arrangement, each group will contain both contracted and non-contracted fibrils at certain phases of the beat, and the two groups will be bound together by the connecting structure between the two central fibrils.

The functions of the basal body are certainly concerned with morphogenesis, in that they are the centres for the organization of both the ciliary shafts and the ciliary roots. They also form a connexion between the ciliary shaft and the root structures. Ciliary roots are responsible for anchorage in many cases, and may also function sometimes in the conduction of co-ordinative impulses; the basal bodies must take part in both of these functions. The excitation of contractions in the ciliary fibrils almost certainly starts in the basal body, although contraction of glycerine extracted models in ATP may take place after detachment of the basal body.

It is interesting that a " basal foot " is found on fibrils 5 and 6 of the basal bodies of *Anodonta* cilia, where the effective stroke is known to be directed towards this pair of fibrils; this foot may be concerned in excitation, and thin fibrils which could function in conduction are seen attached to the foot in some electron micrographs (Pl. IIb). The same cilia show a functional adaptation of the root structure. Since the effective stroke of the cilia, caused by contractions in fibrils 5 and 6, cause them to swing around a fulcrum at the base of the cilium, maximum strain will have to be taken by the roots attached to the bases of fibrils 9, 1 and 2. It is found that a large root is attached to the bases of these three fibrils in the basal body, and smaller roots from fibrils 5 and 6 join the main root at a lower level. When the main root splits into two parts, these diverge in a plane at right angles to the plane of beat, and can take equal strain during the effective stroke.

4. The Mechanism of Beat

Three distinct processes are involved in the beating activity of a cilium, viz. (1) the excitation of contraction, (2) the contraction itself, and (3) the propagation of the contraction along the length of the ciliary shaft. For normal regular beating of a cilium all three are required. The study of glycerine-extracted models of flagella and sperm has indicated that the contractile elements will show regular alternations of contraction and relaxation even after the normal structure has been at least partly disrupted, so that some automaticity seems to be built into the contraction machinery. These models do not show propagation of the contraction. The rate of beat of cilia may be modified by many factors, some of which no doubt have a direct effect on the contractile mechanism, while others may act on the excitation mechanism; the very existence of ciliary control in metachronism and reversal shows that the ciliary contraction machinery is not entirely automatic in its action.

Theories concerning the mechanism of contraction are best considered first, since we can apply biological and mechanical observations on other structures to the known anatomy of the cilium. Older theories involved suggestions that the cycle of movement of a cilium resulted from such things as the flowing of hyaloplasm into and out of a curved hollow outgrowth (Grant, 1835; Schäfer, 1891), or of the rhythmic contraction of the ciliary membrane (Heidenhain, 1907). The suggestion of Sharpey (1835) that cilia possess internal contractility is much more in line with current theories, most of which assume that the peripheral fibrils are capable of shortening in some way.

A large amount of investigation on the mechanism of contraction of muscle, particularly vertebrate striated muscle, has led to the formulation of several hypotheses about the shortening of muscle fibres. It is only reasonable that some attempt should be made to see if the suggested mechanism can be applied to the ciliary fibrils. On the basis of the observed structure and change in striation widths of striated muscle under various conditions, Hanson and Huxley (1955) concluded that there were two types of parallel filament, both rather less than one sarcomere in length, which could be cross-linked and could move longitudinally with respect to one another, both when stretched and in contraction, without change in filament length. That is, contraction is caused by filaments sliding along one another without themselves shortening. Although this hypothesis is attractive and the evidence for it is very strong, not all workers have agreed with it. Sjöstrand and Andersson-Cedergren (1957), for example, have found that the filaments, which are continuous through many sarcomeres, shorten and thicken on contraction, and may interlink and have cross-bridges. Very high magnification electron micrographs by these latter authors show a helical organization of the filaments, apparently built up of very small rodlets; the orientation of these rodlets changes during contraction to become more transverse, so that these authors suggest a folding mechanism rather than a sliding one.

An interesting case of an apparently helical structure which shows similar changes of shape on shortening has been described by Brenner *et al.* (1959) for the tail sheath of the T-even bacteriophages. In the extended condition this sheath is 800 Å long by 165 Å in diameter, apparently in the form of a spiral with 25 turns, while in the shortened condition it is 350 Å long by 250 Å in diameter, and in end-view it now has the appearance of a wheel with 15 cogs. Biochemical evidence suggests that the sheath contains about 200 molecules of about 50,000 molecular weight, so that the shortening of the sheath could result from a change from 25 turns of eight units per gyre to about 14 turns of 15 units per gyre. Note that this structure has a similar diameter to that of a ciliary fibril, and that a change in length actually involves the sliding of one gyre relative to the next.

Several electron micrographs have shown the appearance of a spiral structure in the peripheral fibrils of cilia (p. 19), and it seems reasonable to suggest that the contraction of ciliary fibrils could be the result of a shortening and widening of this spiral. The amount of shortening required is small in cilia compared with that found in isotonic contraction of muscle, e.g. the maximum shortening in the fibrils furthest from the axis of bending in both cilia (Bradfield, 1955) and sperm tails (Gray, 1955) is of the order of 5 per cent, while fibrils nearer to the axis of bending need shorten by less than this in order to produce the observed amount of bending. Fibrils on the outside of the bent region are assumed to be stretched by a corresponding amount, but this depends on the site of the compression elements in the ciliary shaft. The tension that must be exerted in each fibril of the cilium ($2 \cdot 4 \times 10^{-5}$ dyn) to produce the bending couple calculated by Harris (1961) is remarkably close to the force exerted by a single filament of striated muscle in isometric contraction ($3 \cdot 3 \times 10^{-5}$ dyn) (Hanson and Huxley, 1955). However, the ciliary fibril doublets, and even the subfibrils, are much larger than either of the muscle filaments that these authors describe, and in fact the wall of the ciliary fibril may be thicker than the thinner filaments of striated muscle. The figure calculated by Harris takes account only of the external resistance to movement of the cilium. There is no evidence that the two subfibrils of any particular peripheral fibril of a cilium can move relative to each other; indeed, it appears that they both share a common wall along the line that divides them.

Several attempts have been made to explain the way in which the observed bending patterns of cilia and flagella are produced by the shortening of the peripheral fibrils; the ideas put forward by Bradfield (1955), Gray (1955) and Sleigh (1960) all follow similar lines. The model used by Gray to demonstrate his theory is shown in Fig. 41a; it represents a short length of a flagellum in which the central rod *R* is the compression element which prevents shortening, while the peripheral fibrils are capable of shortening by contraction. In Fig. 41b the position of the fibrils is shown in a transverse section of a flagellum in relation to the axes *X*-*Y* and *W*-*V*. Fibrillar contractions are assumed to bend the flagellum about the axis *X*-*Y*, but remain balanced about the axis *W*-*V*. If the tension in fibrils to the right (in the figures) of *X*-*Y* is greater than that to the left, the flagellum will bend to the right and fibrils on the left will be stretched. A bend to the left will similarly result from a greater tension on the left-hand side. It is interesting that if all of the fibrils of the model were to contract simultaneously, and each exerted the same force, the distribution of fibres is such that the resultant bending moment about the line *X*-*Y* would be zero.

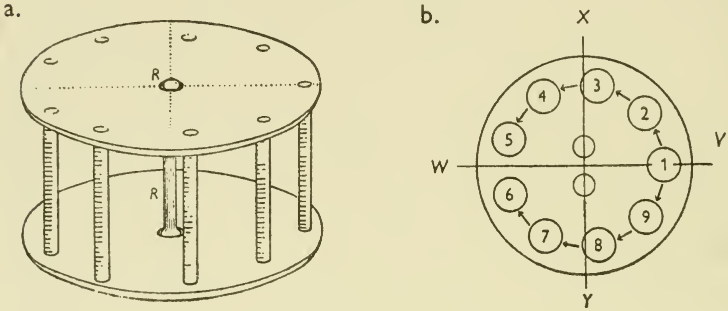


FIG. 41. **(a)** A model representing a short segment of a flagellum (see text) (from Gray, 1955). **(b)** The arrangement of fibrils in a cilium or flagellum (seen looking towards the base) in relation to the axis of bending *X*–*Y* and the plane of beat *W*–*V* (from Sleigh, 1960).

A complete cycle of flagellar bending, moving first to the right and then to the left, could result from a series of contractions in the peripheral fibrils in the order indicated by the arrows in Fig. 41b.

Gray worked out the sequence of bending moments about the line X-Y on the assumption that the fibrils were distributed as shown in Fig. 41b, that they contracted in this order, and that

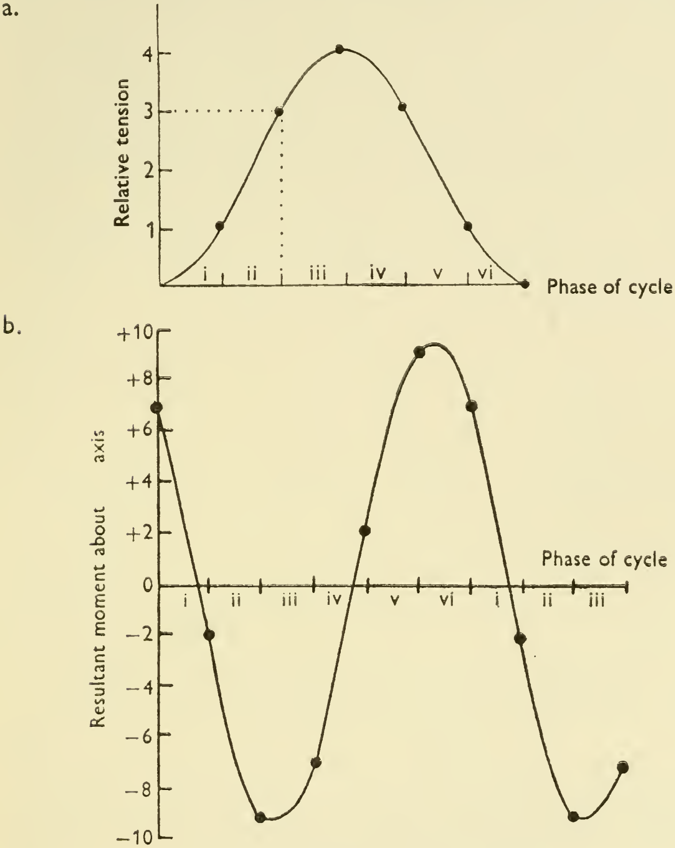


FIG. 42. Graphs illustrating a suggested contraction cycle in the model shown in Fig. 41. **a.** The contraction cycle assumed to take place in each peripheral fibril of the model. **b.** The sequence of resultant bending moments about the line X–Y in one cycle of contraction of the model (constructed using data from **a**) (from Gray, 1955).

the contraction cycle of each individual fibril followed a sine wave (Fig. 42a). Each cycle was divided into six parts, so that each fibril was $\frac{1}{6}$ cycle out of phase with each of its neighbours (except fibrils 5 and 6). The resulting graph, Fig. 42b, follows a sine wave in which one complete cycle of flagellar bending occurs in each cycle of fibrillar contraction, so that the model would bend first to the right and then to the left before completion of the cycle. By this means Gray was able to explain the symmetrical bending waves he found in some sea urchin sperm tails.

An explanation of asymmetrical bending waves using these same assumptions is less easy unless one also assumes that the ability to contract is greater on one side than the other. A small modification of Gray's theory suggested by Sleigh (1960) may provide a more satisfactory explanation of the various forms of ciliary and flagellar beating.

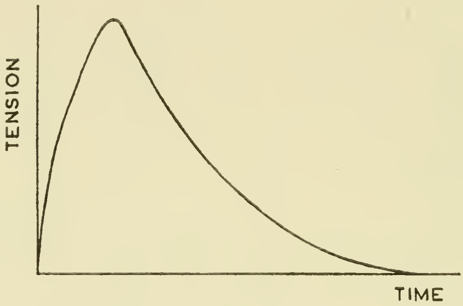


FIG. 43. Diagram of the course of contraction in a muscle fibre. Values from this curve were used to calculate fibrillar tensions in the construction of Fig. 44.

The contraction cycle in a muscle fibre normally follows an asymmetrical course, as in Fig. 43. If we assume that the contraction in each ciliary fibril follows a curve like this rather than a sine curve, then a symmetrical bending cycle of the model would still be produced when each fibril is $\frac{1}{6}$ cycle out of phase with its neighbours, as in Fig. 44a. If the phase difference between adjacent fibrils is greater than one-sixth, the bending cycle of the model will be asymmetrical or reduced in amplitude or both. If the phase difference is reduced to $\frac{1}{12}$ cycle, the bending cycle is moderately asymmetrical (Fig. 44b), while if it is $\frac{1}{24}$ cycle, the bending cycle is very asymmetrical (Fig. 44c).

It is assumed in all cases that the fibrillar contraction lasts about the length of the full cycle, so that a second contraction takes place when the first has completely died away. Even fairly large errors in this assumption will not affect the patterns produced very much.

The model used represents a short length of the flagellum, and a complete flagellum may be built up from a large number of these models placed end-to-end. Contractions in the peripheral fibrils will be propagated up the length of the flagellum from base to tip, activating each unit in turn, so that each unit will be slightly out of phase with neighbouring units. If the bending cycle of each unit is symmetrical, the bending cycle of the whole flagellum will be symmetrical, while if the bending cycle of the component units is asymmetrical, the bending cycle of the whole flagellum will be asymmetrical and that of a cilium, for we have seen that the beat of a *Stentor* membranelle is like a very asymmetrical flagellar beat. The same contractile machinery, acting

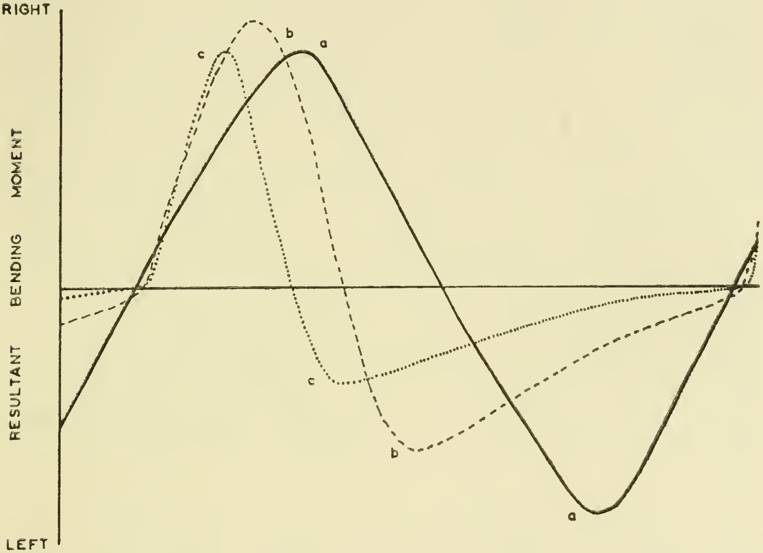


FIG. 44. Graphs showing the calculated sequence of resultant bending moments (about the line X–Y in Fig. 41b) in cilia where the phase difference between adjacent fibrils is **(a)** $\frac{1}{6}$, **(b)** $\frac{1}{12}$ and **(c)** $\frac{1}{24}$ cycle (see text).

in the same way both as regards its contraction and the propagation of the contraction, could produce both the flagellar and ciliary beating patterns.

It is assumed in this hypothesis that the cycle of beat starts with a contraction in fibril 1, and that contraction of the other fibrils follows in the order indicated by the arrows in Fig. 41b. This is in accordance with the finding by Afzelius that the effective stroke is normllay towards fibril 1 in *Mnemiopis* cilia (p. 17), but in *Anodonta* cilia Gibbons has neatly shown that the effective stroke is towards fibrils 5 and 6. It is interesting that *Mnemiopsis* comb-plates are capable of reversal, and may therefore start their beat towards either fibril 1 or fibrils 5 and 6. Cilia of *Opalina* may beat in any direction, and presumably any fibril, or pair of fibrils, may be the first to contract.

If curves similar to those in Fig. 44 are constructed on the assumption that contractions in fibrils 5 and 6 start the beat, then results very close to those shown are obtained, being almost identical for the symmetrical beat (a), and showing slightly increased asymmetry in graphs b and c. Valuable evidence for the theory is provided by the findings of Afzelius (1959) and Gibbons (1961b) that a permanent bridge structure connects fibrils 5 and 6 in sea urchin sperm and *Anodonta* cilia; this suggests that these two fibrils always move together. Similar links are not found between other fibrils in the main part of the shaft.

According to the theory outlined above, the various beating patterns found in cilia and flagella differ primarily in the timing of the contractions in the component fibrils. That is, the ratio :

$$\frac{\text{Interval between the excitation of neighbouring fibrils}}{\text{Interval between successive excitations of the same fibril}}$$

(which is equivalent to the phase difference between adjacent fibrils) determines the form of beat. If the phase difference is greater than one-sixth, the resultant bending moments are reduced since contractions on the two sides will always oppose one another, e.g. at a phase difference of one-fifth the maximum bending moment is little more than half that at one-sixth, although the bending cycle is reasonably symmetrical. If the phase difference is less than one-sixth, the bending cycle is asymmetrical in such a way that the bending to one side is nearly maximal, but continues for a smaller part of the complete cycle, while the bending to the other side is much less and continues for a longer time. All gradations are possible, therefore, from a symmetrical flagellar beat to an extremely asymmetrical ciliary beat, merely on the basis of a change in phase difference between the contraction of neighbouring fibrils of the peripheral ring.

It may be possible one day to test this idea, for many factors may vary the phase difference, either by their effect on the contraction or excitation processes, but we do not yet know enough about the normal beat in most cases to be able to detect anything but a major change in the form of beat. It would be most instructive to consider those factors which destroy the symmetry of beat of a sperm tail; perhaps someone will soon study this, for at present we only know that the beat tends to become more asymmetrical with age (Gray, 1955), a result that might be expected if the contraction was slowed while the excitation between fibrils remained at the same rate.

The actual time required for the proposed fibrillar excitations seems reasonable for both sea urchin sperm tails and *Stentor* membranelles. If the phase difference is about one-sixth in symmetrically beating sperm tails, and each bending cycle takes about 30 msec, the excitation interval between neighbouring fibrils is about 5 msec. In *Stentor*, if the phase difference is about one-twenty-fourth (as in Fig. 44c), and the time for one beating cycle is 36 msec, then the excitation interval would be 1·5 msec. A parallel case in nerve physiology might be the time delay during excitation at a nerve synapse, where an interval of 1 to a few msec is usual. Few cilia will show both a faster beat and a more asymmetrical beat than *Stentor*, so that we should not expect excitation intervals of less than about 1 msec, although those of some slowly beating cilia may be much longer.

The mechanism of propagation of the contraction wave along the fibrils is less amenable to this sort of approach, but some suggestions made by Harris (1961) make a good start. The rate of propagation of contraction under normal conditions seems to lie in the range between 100 and 1000 μ/sec (see Table 12). As Harris pointed out, such a speed is far too low to be caused by the purely mechanical means concerned in the propagation

TABLE 12. THE RATE OF PROPAGATION OF THE CONTRACTION WAVE IN SOME CILIA AND FLAGELLA

Organelle	Frequency beats/sec	Cilium length (μ)	Rate of propagation (μ/sec)	Temp. (°C)	Reference
Sea urchin sperm	30–40	40	800–1000	18·0	Gray (1955)
Bull sperm	9·1	(60)est.	(400–500)est.	37·0	Gray (1958)
Peranema flagella	5–6	80–100	*c.*200	?	Lowndes (1941)
Euglena flagella	12·7	128	813	?	Lowndes (1941)
Stentor membranelle	27·7	30	730–920	18·5	Sleigh (1960)
Opalina cilia	1–4	10–20	20–100	17–18	Sleigh (1960)
Mytilus abfrontal cilia	2–3	100	500	15	Gray (1930)
Mytilus lateral cilia	16	10–20	(160–320)est.	15	Gray (1930)
Grantia flagellum	5–20	30	25–500	?	Bidder (1923, 1927)
Paramecium cilium	28	12	350	*c.*20	Sleigh (unpub.)

est. = estimated.

of a wave in an elastic rod. In terms of biological processes, the transmission is also too slow to be compared with nerve conduction, but bears closer comparison with the transmission of the wave of contraction in muscle fibres. Harris suggested that the rate of conduction of the contraction in muscle fibres has a maximum value of about 1 mm/sec, and he quotes examples from muscles as diverse as the striated wing muscle of insects (where the unit is the sarcomere), the body wall muscle of *Ascaris*, the stomach wall muscle of the cat, the slow adductor and the byssus retractor muscles of *Mytilus*. If either muscles or cilia are under restraint the conduction may be slower than this. It seems then as if there is an intrinsic conduction mechanism in these contractile structures.

If we consider the propgataion of a contraction wave along a single fibril, a contraction in one region will be expected to stretch an adjacent uncontracted region, and it would seem reasonable to follow Gray (1955) in believing that the release of tensile energy follows mechanical stretching. This stretching could uncover active ATP-ase sites, which could cause the release of energy for contraction in the same manner as has been suggested for muscle. In the whole cilium, however, the fibrils on the opposite side of the shaft are stretched at least as much as the adjacent parts of the same fibril, yet they do not contract. This may be because fibrils on the convex side of the bend in the shaft have not completed their contraction cycle and are still refractory. Harris has suggested that this idea should be abandoned in favour of the assumption that it is shortening which excites contraction, while stretching inhibits it. There is some evidence that shortening of muscle may cause activation, e.g. in the rhythmic contraction of the tymbal muscle of the cicada (Pringle, 1954), although most workers have assumed that stretching precedes contraction. Observations on the effects of high viscosity of the medium on *Stentor* membranelles gives evidence in favour of the shortening theory, for a cilium may be excited to beat by mechanical drag communicated through the viscous medium. In this way the bending of one cilium assists that of its neighbour, and it appears that contraction is excited by the mechanical initiation of a slight bend at the base of the neighbouring cilium before the normal metachronal excitation is complete. The normal bending cycle is not shortened in the viscous medium, because only in the effective stroke can cilia assist each other, while in the longer recovery stroke the ciliary movements will be slowed by the increased viscous resistance. In fact, the bending cycle takes about the same time in viscous media in spite of the fact that the amplitude is reduced.

In the bending cycle of a symmetrically beating flagellum, a fibrillar contraction follows the return to normal length after stretching of the fibril, i.e. it immediately follows shortening, and it is easy to visualise the way in which shortening could excite contraction. Much the same may happen in the recovery phase of ciliary beating, for, as Harris wrote, " distal to each element of the advancing contraction on the effective side will be a region that is bent into a concave curve "; this bending could excite the fibrils by their shortening on that side. Fibrils on the opposite side may be excited as in flagella. The bent region is more or less limited to the basal region of the cilium for most of the effective phase, and the bending wave only starts to move up the ciliary shaft towards the end of this period. In cilia that are short (or compound cilia of greater length) the effective stroke is quickly completed and the bending wave soon spreads up the cilium, but in longer cilia the effective stroke may take longer, so that some time elapses before the bend at the base is complete and contraction spreads up the cilium. The shortening theory of propagation of the contraction explains this adequately, for only towards the end of the effective phase will the tension in fibrils on the effective side of the cilium be released sufficiently for the fibrils to shorten and propagation of the contraction to take place.

If such a simple mechanical explanation is correct, it is surprising that glycerine extracted models do not show propagation of the contraction, although it is possible that in this case the contractions are not localized, but extend for the whole length of the cilium. Some further observations on these models may be very valuable in this respect.

The propagation of the contraction is evidently closely associated with the contraction itself, for environmental changes which affect the frequency of beat also seem to affect the rate of propagation. For example, in viscous media the rate of propagation is slowed by about the same amount as the frequency, while in magnesium chloride the propagation rate is speeded up by about the same amount as the frequency. If both of these factors affect the rate of shortening of the ciliary fibrils, either by increasing external resistance or decreasing internal resistance to movement, and if the propagation is mechanical, these are the results that we should expect. Additional evidence for the dependence of propagation on contraction is provided by the fact that the rate of propagation usually increases as the wave of flexure moves towards the tip, where the resistance to movement is lessened.

Some mention of the excitation of contraction has already been made above. It is known that glycerine-extracted models will contract alternately on the two sides in the presence of ATP, and that cilia can be mechanically co-ordinated in very viscous media. In both cases a shortening of the fibrils, either following stretching or because of viscous drag, could be responsible for exciting the new contraction of the cilia. While some cilia may normally be excited to beat by mechanical means, many cilia may be co-ordintaed metachronally by some internal mechanism which is most likely to act by excitation of the cilia (see p. 184). We are ignorant of the mechanism of excitation. It is tempting to suggest that it is connected in some way with the provision of suitable conditions for the breakdown of ATP to release the energy for contraction, and that this could result either by some structural change (shortening ?) or by some chemical change, the latter perhaps concerned with some change in ionic concentrations. Of the drugs which increase the rate of transmission of metachronal waves, both serotonin and veratrine also increase the rate of beat (i.e. the rate of excitation) considerably, and the latter drug is thought to act by an effect on the ionic balance across the cell membrane. It is difficult to visualize any means by which the excitation may be restricted so that only one fibril is excited initially, unless it be assumed that this fibril has the lowest threshold, or that other fibrils are refractory. The " basal foot " on the ciliary basal body of *Anodonta* may provide a specific site of excitation in this case, but such structures are not widely known. Another problem concerns the way in which reversal of beat fits into this picture, unless the changes that cause reversal specifically favour the excitation of other fibrils in the ring.

5. The Energy Relationships of Cilia and Flagella

Evidence has been presented that the energy for the beating of cilia is derived from glycolysis and the citric acid cycle, and is carried to the site of utilization by the high energy phosphate bonds of ATP. The nine peripheral fibrils of cilia have been found to carry ATP-ase activity. Estimates of the energy available for use in the movement of cilia or flagella can be based on observed chemical changes, and also estimates of the energy required in this movement can be based on theoretical calculations and in some cases on actual measurements.

The energy for movement of bull sperm under anaerobic conditions may be obtained by the breakdown of exogenous fructose to lactic acid. Rothschild (1961) was able to measure the rate of fructolysis by bull sperm, and, knowing the free energy change associated with the reaction, he found that at 37°C the sperm were able to release energy at a rate of $6 \cdot 10 \times 10^{-6}$ erg/sperm/sec.

The rate of energy expenditure in overcoming the resistance of external viscosity in the movement of sperm can be calculated from a theoretical equation quoted by Rothschild (1961); in this calculation the energy used along the whole tail is integrated. By substitution of measured values from bull sperm in this equation, it was found that each bull sperm uses $2 \cdot 11 \times 10^{-7}$ erg/sec (at 37°C) in movement. This is near one-thirtieth of the energy available from fructolysis. On the assumption that the energy for movement is carried by ATP and released by ATP hydrolysis, Rothschild found that the energy available for movement could not exceed $1 \cdot 8 \times 10^{-6}$ erg/sperm/sec, which still leaves a factor of about 10 between the energy available and that required. Similar calculations on the sea urchin sperm gave a figure of $2 \cdot 10 \times 10^{-7}$ erg/sperm/sec for the energy dissipation in movement at 17°C. In both sea urchin and bull sperm only the work done against external resistance has been considered in the theoretical calculations, the energy required to bend or compress elements of the tail is not known at all.

Other data on the bull sperm are available from the work of Nelson (1958), who found by the use of a formula of Carlson that each sperm dissipates energy at a rate of $3 \cdot 15 \times 10^{-8}$ erg/sec. He also found from biochemical studies that enough succinic dehydrogenase was present in sperm tails to regenerate some 20 times the ATP required for this (bull sperm may use the citric acid cycle to provide free energy).

It is not so easy to study cilia in this way, but both practical and theoretical figures are available. According to Jensen (1893) each cilium of *Paramecium* is able to exert a force on the water of about $4 \cdot 5 \times 10^{-7}$ mg. If this force is exerted in each effective stroke, then the rate of doing work in the effective stroke is of the order of 1×10^{-7} erg/cilium/sec, and the average rate of energy consumption throughout the cycle is probably rather less than this.

The result of calculations based on the figures used by Harris (1961) for *Paramecium* may be compared with this. If the cilium of *Paramecium* exerts a bending couple of 5×10^{-10} dyn cm in order to overcome viscous resistance in the effective stroke, and the cilium moves through an angle of rather more than 90° in this phase of beat in a time of about $0 \cdot 008$ sec, work is done at a rate of about $1 \cdot 25 \times 10^{-7}$ erg/cilium/sec in the effective stroke. Here again, the average rate of doing work is likely to be rather less than that found for the effective stroke alone. The figure obtained in this calculation agrees well with that obtained from Jensen's data, and tolerably well with the figures for the utilization of energy by sperm calculated by Rothschild.

Compound cilia may be treated in much the same way. Thus, the bending couple calculated for a membranelle of *Stentor* at about 20°C (see p. 147) is about 3×10^{-8} dyn cm, and the membranelles move through about 140° in the effective stroke, so that the work done in the effective stroke is about 7×10^{-8} erg/cilium/beat. These compound cilia beat at about 30 beats/sec and each effective stroke occupies about a quarter of the beat, which means that the rate of doing work in the effective stroke is about $8 \cdot 4 \times 10^{-6}$ erg/cilium/sec. Since 60 to 75 cilia are present in each membranelle, each component cilium uses energy at a rate of $1 \cdot 1$ to $1 \cdot 4 \times 10^{-7}$ erg/sec in overcoming external resistance in the effective stroke.

A direct measurement of the force exerted on a needle which arrests an abfrontal cilium of *Mytilus* has been made by Yoneda (1960). Very fine glass microneedles were prepared, and the force required to displace the tip of each needle by 1 μ was measured. These needles were used to arrest the cilia near the start of the "effective stroke" by holding them at various distances from the ciliary base. The mean torque around the base of these cilia found by this method was $3\cdot9 \times 10^{-7}$ dyn cm in cilia of mean length 54·4 μ. The variation in torque lay between 2×10^{-7} and 8×10^{-7} dyn cm for cilia between 42 μ and 66 μ in length at temperatures between 18 and 27°C.

It has already been pointed out that these cilia are compound structures, but it is not yet known how many component cilia are involved. It is also important that these cilia show an abnormal beat that deserves further study (p. 143). If the torque measured were to be exerted throughout the effective phase of beat, and the angle of beat is a little over 90°, the work done in each effective stroke is about 8×10^{-7} erg. The effective stroke occupies nearly $\frac{1}{2}$ sec, so that the compound cilium is doing work at a rate of about $1\cdot6 \times 10^{-6}$ erg/sec in this phase of beat.

No attempt at calculation or measurement of the work done against the internal resistance and stiffness of the cilium has yet been successful. The energy available for use in locomotion of bull sperm is some 10 times that required to overcome the external resistance to movement of the sperm tail; it seems unlikely that the work required to overcome internal resistance would use all of the remaining energy that is available.

It is interesting that flagella probably show a fairly uniform expenditure of energy throughout their length, while cilia probably use more energy in the effective stroke than in the recovery stroke, and so most of the energy used by cilia goes to contraction of the basal parts of the ciliary fibrils. Energy in convenient form may be provided from mitochondria which usually lie near the bases of cilia, or may also, it appears, be released nearer to the site of utilisation. Sperm frequently carry mitochondria around the neck (e.g. sea urchin) or the mid-piece (e.g. bull). While both sea urchin and bull sperm may use energy at about the same rate to overcome viscous resistance at their normal temperatures, the bull sperm move at rather less than half the speed of the sea urchin sperm, and have a much larger energy producing system in their mitochondria. The bull sperm also has to move a larger head through the fluid medium, and has to bend larger sheath structures in the tail.

6. The Determination of the Rate of Beat of Cilia

From a physical point of view, the rate of beat of a cilium must depend on its size, both as regards its length and its compound nature; the width of single cilia is fairly uniform, and such differences as are found probably make little difference to external resistance to movement, although internal resistance may be changed. Harris (1961) has put forward the idea that if one assumes that the bending force that can be exerted by cilia is constant, the duration of the effective stroke is proportional to the cube of the cilium length (at constant amplitude), while the duration of the recovery stroke is proportional to the cilium length. If the cilia are compounded together, the duration of the effective stroke is inversely proportional to the number of component cilia, while that of the recovery stroke is unaltered.

Any particular cilium may vary in its rate of beat because of changes in the viscosity of the medium which change the external resistance to movement of the cilium. It is possible that changes in the internal resistance to ciliary bending may also take place. The rate of propagation of the bending wave along a cilium may also be influenced by the viscous resistance to flexure of the cilium. Purely physical factors which affect the movement of a cilium may thus influence the rate of beat, presumably by affecting the rate at which it can contract. It is conceivable that in some cases the rate of beat is limited by the availability of energy for contraction. Some workers have found that the beating of cilia may be accelerated by addition of ATP; this may be acting as an additional source of energy, or could conceivably facilitate ciliary movements by a plasticizing action.

Under most circumstances cilia seem to be subject to excitation, either intrinsic or extrinsic, and the rate of excitation of cilia may be the factor which normally determines their rate of beat. Most cilia that have been studied may be made to beat faster by the application of drugs like serotonin, adrenalin or veratrine, and in all three cases the drug may act on the excitation of the cilia.

The rate of beat may thus depend on either the rate of contraction or the rate of excitation, for it appears that either may be limiting. It is not known for certain whether one beat of a cilium must always be completed before the next one starts. It appears from some observations e.g. the beat of *Sabellaria* cilia, Fig. 40a, that a second beat may commence before the first bending wave has reached the tip. Even if this is general, the rate of contraction could still limit the rate of beat. Much more information about the ciliary mechanism must be obtained before such ideas can be extended or confirmed.

7. Summary

The beating patterns of flagella appear to be simpler than those of cilia, although the same mechanism can account for the movements shown by all organelles. In the " ideal " flagellar beat, sinusoidal waves of bending take place in a single plane and are propagated along the length of the flagellum from base to tip. This symmetrical bending is the result of equal and alternate contraction waves passing along the two sides of the flagellum. In most cases the flagellar beat departs from this ideal in that the rate of propagation of the bending waves along the flagellum changes as the waves pass along the flagellum, and frequently the bending on the two sides of the flagellum is unequal. Inequality of bending is thought to result from inequality of time intervals between the bending movements on the two sides of the flagellum (i.e. the intervals between ' right ' and ' left ', and between ' left ' and ' right '); in the more extreme cases of asymmetrical bending, the movements that result are typical of the beat of cilia. It is not always clear whether a structure is behaving as a flagellum or a cilium, for all intermediates between a truly symmetrical and a very asymmetrical beat may be found. Symmetrical and asymmetrical patterns of movement have been described in this chapter.

There is some support for the belief that the movements of cilia and flagella are the result of localized contractions of the internal fibrils, and that these contractions are propagated throughout the length of these organelles. It seems most likely that shortening of the nine peripheral fibrils is responsible for bending of the ciliary shaft. Rigidity of the cilium could not be maintained by the two central fibrils alone, and it is suggested that either internal turgor pressure acting against the elastic tension of the ciliary membrane, or the cross-linked scaffolding of central and peripheral fibrils, could maintain the shape of a cilium against distortion by bending. In addition to their function of morphogenesis, the basal bodies of cilia are the site of origin of waves of contraction which pass up the cilia; they are also responsible for anchorage since they connect the ciliary shaft to the root structure.

The beating of a cilium involves three distinct processes: excitation of contraction, contraction and propagation of the contraction. Although shortening of the fibrils in contraction could occur by the sliding of longitudinal filaments alongside one another, it seems more likely that the peripheral fibrils may be composed of filaments arranged in helices, and that the fibrils shorten and thicken by the sliding of one gyre of the helix relative to the next. The fibrillar shortening required is small compared with that in muscle, and the proposed contraction system would be adequate. The mechanism of the propagation of contraction waves seems likely to be mechanical rather than electrical, and to involve only the peripheral fibrils of the cilium; the suggestion that contraction follows shortening of the fibrils seems to be more plausible than the theory that stretching must precede contraction. Very little is known of the mechanism of excitation; some connection with chemical, particularly ionic, changes is likely, but mechanical excitation is also believed to occur.

Some calculations of the requirement of energy to overcome the external resistance to movement of several types of cilia and sperm tails have been made; these show reasonable agreement with each other, and some also show agreement with the calculated rate of utilization of ATP by the structures concerned. The availability of energy may affect the rate of beat of a cilium, or the rate of beat may be limited by the rate of excitation or the external resistance to bending.

CHAPTER 5

THE CO-ORDINATION OF BEAT OF CILIA

1. Introduction

Where many ciliary organelles occur together, an economical use of their activity can only be achieved if the movements are organized in some way. Organelles with a typical flagellar beat are usually found singly or in pairs on single-celled bodies like spermatozoa or the simpler flagellates, while, in those organisms where many flagella are present, the arrangement and attachment of these flagella is such that they all work together in that they cause movements of water in the same direction relative to the body. In sponges, for example, the arrangement of the flagellated cells is such that flagellar beating causes a current of water to pass through the sponge in one direction only, and there is no possibility of change in beat direction or direction of water current, only stoppage. Similarly, in the trichonymphid flagellates, the flagella are inserted on the body in such a way that their free ends are directly posteriorly. In *Pseudotrichonympha* the basal regions of the flagella are even attached to the walls of grooves on the body surface to keep them in position, and Gibbons and Grimstone (1960) noticed that, although the basal bodies are nearly perpendicular to the body surface, the flagella may bend fairly sharply at the body surface; they also found that in *Holomastigotoides* the basal $0 \cdot 8\ \mu$ of the flagellum " appears to be closely applied to the body surface ". The beating of these flagella always moves the water posteriorly over the surface of the animal, and all the flagella work together in the sense that all are similarly orientated and all move the water in the direction from flagellar base to tip. Groups of flagella like this may still be subject to control by inhibition, but it is doubtful whether they are coordinated to beat rhythmically by any means other than viscous interaction.

If many cilia are grouped together, then their beating requires more careful organization because of the fact that the beat is specialized to move water in a direction at right angles to the longitudinal axis of the cilium. Mere parallel alignment of cilia is not sufficient to ensure that they all work together, as it would be for flagella, for the ciliary beat must also be lined up so that all the members of a group beat in the same plane and in the same direction.

The force of the ciliary beat can be used more efficiently if cilia which beat in the same plane and in the same direction are made to beat synchronously. However, a synchronous beat is apparently unsuitable for use over a large area of ciliated surface, perhaps because it gives a jerky movement rather than a steady progression of water over the ciliated surface, or perhaps because it is not practicable to excite a synchronous beat over a large area. In place of overall synchrony it is found that there are two well-defined lines at right angles to each other in any active ciliated epithelium, one in which all the cilia beat together, and the other in which they beat in succession to give the appearance of waves called "metachronal waves". It is usual to find that the bases of the cilia are arranged approximately in rows in these two directions; Knight-Jones (1954) has named these "*orthoplectic*" rows—in line with the beat—and "*diaplectic*" rows—at right angles to the beat.

2. The Compounding of Synchronously Beating Cilia

Cilia of short orthoplectic rows, e.g. the membranelles of *Stentor* and the laterofrontal cilia of *Mytilus* gills, of diaplectic rows, e.g. the comb-plates of ctenophores and perhaps the undulating membrane of some ciliates, and occasionally groups of more or less isolated cilia, e.g. cirri of *Euplotes* or abfrontal cilia of *Mytilus* gills, may have their bases packed closely together and their shafts united to form compound structures (p. 30).

The compounding of such synchronously beating cilia has a functional advantage which was pointed out by Harris (1961) (see p. 146). The angular velocity with which a cilium can move is directly proportional to the bending couple that the cilium can exert, but inversely proportional to the cube of the cilium length, and hence the velocity with which the fluid can be moved by the cilium (proportional to angular velocity × length) is inversely proportional to the square of the cilium length. If each simple cilium is capable of exerting about the same bending couple, there is an optimum length for any type of cilium which depends on its use and form of beat. If a cilium is short, it is unable to sweep through a large body of water, but yet can beat very fast, while a long cilium can move through a large volume of water at a much slower speed; the optimum length is attained when the cilium can move through a sufficiently large arc to perform the required function, but yet is able to beat fast enough to perform this function properly.

On this basis Harris suggests that it would be valueless to the animal if the body cilia of *Paramecium* or the cilia of the pharyngeal epithelium of the frog were to be increased in length much beyond 10 to 15 μ, since in both cases the cilia are used to move fluids in the immediate neighbourhood of the surface; increase in length would mean that the cilia would beat more slowly and the movement of fluid would be reduced. Where a longer cilium is required to move through a large volume of water, e.g. for the movement of ctenophores or for the feeding current of *Stentor* or *Mytilus*, then a high velocity of the beat can only be maintained if a number of cilia beat together to increase the bending couple. The effect of this is most easily seen in compound cilia, but it may be used in cilia which are close together and beat synchronously so that the effects of viscous drag may be minimized. Compound cilia used as " legs ", e.g. the cirri of hypotrich ciliates, are also able to make use of the larger bending couple that can be exerted when many cilia beat as one.

A striking example of the effect of cilium length and compounding on the rate of beat is provided by the comb-plates of ctenophores. If a single cilium of such a comb-plate is 1 mm long, and has a bending couple comparable with that of other cilia, then according to the equation worked out by Harris, the angular velocity of beat would be so slow that the cilium would take several hours to complete a beat of the normal pattern. However, in the normal comb-plates, some 10^5 cilia are combined together into a single structure, so that the bending couple that can be exerted is large enough for the comb-plate to beat several times per sec. Note that this is a hypothetical example, in the sense that the excitation and contraction times of a single cilium would not permit a single contraction to occupy such a long time.

The cilia of many epithelia are not compounded, but it is probably important that the synchronous beat of a number of cilia in the direction at right angles to the metachronal waves must be advantageous in that the viscous drag on each cilium is reduced (provided that the cilia are near enough to each other), and the fluid can be moved over the surface faster than it could if the beat were asynchronous.

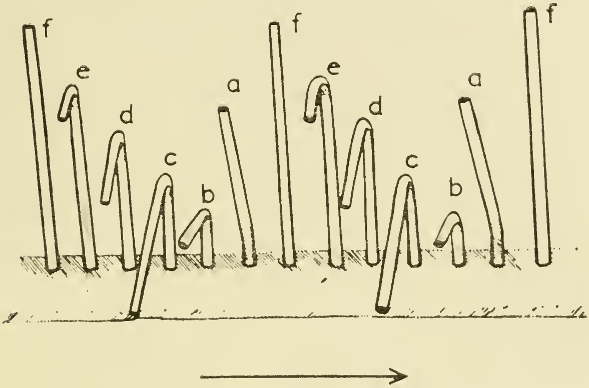


FIG. 45. Diagram showing two metachronal waves of *Stentor* membranelles. The compound cilia are beating in a direction directly towards the observer, and the metachronal waves are moving from left to right (see text).

3. Types of Metachronal Pattern

The metachronal waves that are seen to pass across any ciliated epithelium result because each cilium of the wave is at a slightly different stage in its beat from its neighbours that lie along the line of wave transmission. For example, a cilium may be at the beginning of its effective stroke, while the cilium before it in the wave has started its effective stroke and the cilium behind it has not completed its recovery stroke. Two metachronal waves of the type shown by the membranelles of *Stentor* are represented diagrammatically in Fig. 45. At *f* the cilium is at the end of the recovery stroke, it then bends at right angles to the plane of the page in the effective stroke to pass through stage *a* to *b*, after which the bending wave is propagated up the cilium in the recovery stroke *b* to *f*. In the diagram each of the 6 stages shown is $\frac{1}{6}$ cycle out of phase with each of its neighbours. Note that while it is possible to follo twhrough the stages of beat by moving along the row from right to left, the metachronal waves move along the row from left to right, since *f* is the next cilium to be excited and *a* has already started to beat. Here the cilia at the end of the recovery stroke form the wave crests, and those at the end of the effective stroke form the troughs of the waves. The appearance of waves on other ciliated surfaces will vary according to the pattern of metachronism and the form and amplitude of beat.

Synchronous beating of cilia may occur in either the orthoplectic or diaplectic rows, and consequently, metachronal whves may travel along either diaplectic or orthoplectic rows, since the plane of beat and the direction of propagation of the metachronal waves may coincide or be at right angles to each other. There are four alternative patterns of relationship between the beat direction and the direction of wave transmission, all of which occur widely in nature, although their distribution is little studied yet. Knight-Jones (1954) named these four patterns as follows: *symplectic metachronism*, where the direction of beat and the direction of wave transmission coincide; *antiplectic metachronism*, where these two directions are opposite; *dexioplectic metachronism*, where the effective beat moves to the right with respect to the direction of wave transmission; and *laeoplectic metachronism*, where the effective stroke moves to the left with respect to the direction of wave transmission. These four patterns are illustrated in Fig. 46; some examples are listed in Table 13 (p. 181), and a few of them are described in detail below. Where the metachronism travels along the diaplectic rows (i.e. in dexioplectic or laeoplectic metachronism) the beat takes plcae at an angle of about 90° to the diaplectic row, though it may vary from this by a few degrees either way. In any particular ciliated tissue, the pattern of metachronism is constant, and, where reversal of beat has been reported, the metachronal wave propagation seems to reverse also.

The most familiar pattern of metachronal waves is probably the symplectic one, since the analogy of the appearance of metachronal waves to the waves made by the wind blowing over a field of corn usually refers to this pattern (although Sharpey (1835) used it to describe the diaplectic metachronism of rotifers). This is perhaps unfortunate, since the symplectic pattern seems to be relatively rare. Symplectic waves are easily seen if *Opalina* is examined under dark-ground illumination. Okajima (1953) and Sleigh (1960) have described the pattern of waves, and the structure and arrangement of the cilia in longitudinal rows is well-known from the work of Pitelka (1956) and Noirot-Timothée (1959). The waves normally travel from the anterior end backwards, so that any cilium is in phase with all cilia in the same transverse line, but at a different stage of beat from neighbouring cilia in the longitudinal direction. Cilia beat at right angles to the wave front, and, since the effective stroke is in the same direction as the transmission of the metachronal waves, the wave crests are composed of many cilia moving together in their effective stroke (Fig. 47). Because of this, cilia are unlikely to exert much effect on the water individually, but the movement of the whole wave over the body surface as a fairly solid ridge will move the water quite effectively. Since cilia

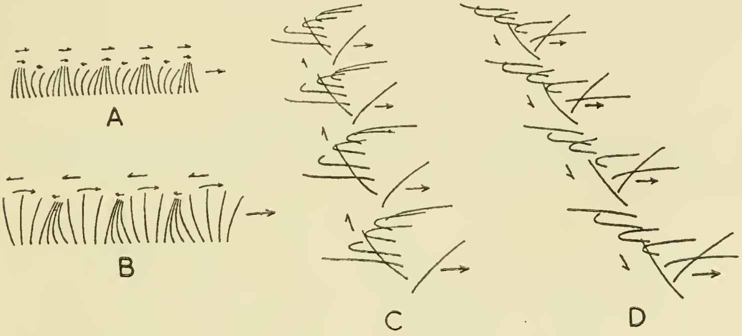


FIG. 46. Diagrams illustrating the four main patterns of metachronal co-ordination. In all the diagrams the effective stroke of the beat is towards the right, as indicated by the complete arrows. The direction of movement of the metachronal waves is indicated by the single-barbed arrows. The patterns shown are: **A,** symplectic; **B,** antiplectic; **C,** dexioplectic; and **D,** laeoplectic (from Knight-Jones, 1954).

naturally work together in this type of metachronal pattern, compounding of the cilia is unnecessary, and co-ordination by mechanical interaction is possible (see p. 183). The direction of transmission of the metachronal waves in *Opalina* is not fixed, but changes rapidly so that the waves move transversely across the animal or even pass forwards (Fig. 53, p. 194, and Pl. XVIIIa); the direction of beat always changes at the same time, and the metachronism is always symplectic. The problem of the change in beat direction is discussed separately (p. 192).

A well-known example of antiplectic metachronism is provided by the rows of comb-plates of ctenophores. In *Pleurobrachia*, for example, the metachronal waves normally travel from the aboral pole of the animal towards the oral end, while the effective stroke of the beat is towards the aboral pole, so that the animal moves mouth forwards. During the effective stroke the comb-plates are spread out like the spokes of a paddle wheel, moving separately through the water and obtaining the maximum effect from the broad surface of the comb-plate (Fig. 48). It is evident that the compounding of cilia is here of a considerable advantage because each comb-plate works alone, the larger it is and the larger the force it can exert, the more effective it is in the movement of water. Here compounding of long orthoplectic rows would not be practicable, though the comb-plates in fact consist of 80 to 100 cilia in the orthoplectic direction and

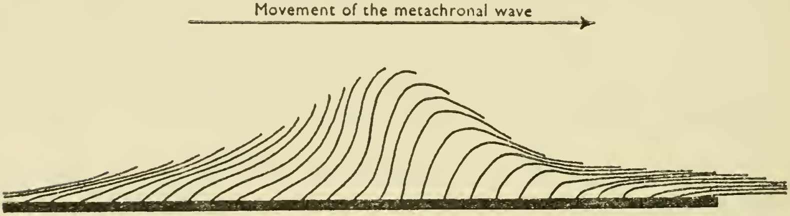


FIG. 47. Diagram of a metachronal wave of *Opalina* showing the component cilia. The plane of beat is approximately in the plane of the paper, and successive movements of a cilium can be followed by moving along the row from right to left (cf. Fig. 39 and Plate XVIIIb). The animal is moving towards the left (from Sleigh, 1960).

up to several thousand in the diaplectic direction, all tightly compressed together. In other uses of this type of metachronism compounding of cilia may not be necessary. Coonfield (1936) describes the reversal of the metachronal waves of *Mnemiopsis* in feeding, and, to judge by the movements of feeding ctenophores described by Hardy (1956), the reversal of beating of ctenophore comb-plates must play an important part in ctenophore behaviour. It appears that when the wave is reversed, the beat is also reversed.

Diaplectic metachronism is very widespread, especially in metazoa, and Knight-Jones (1954) has found that the distribution of dexioplectic and laeoplectic metachronism is of phylogenetic interest in that both types are seldom found in the same group. In the protochordates, for example, diaplectic waves always show

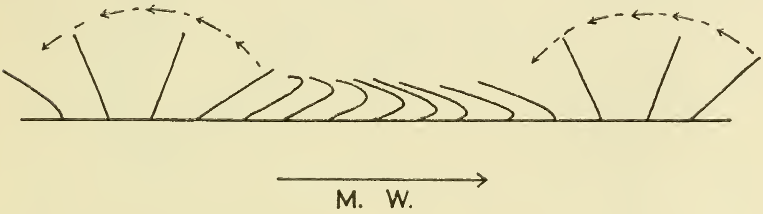


FIG. 48. Diagram of metachronal waves of comb-plates of the ctenophore *Pleurobrachia*. The effective stroke is towards the left and the metachronal waves move towards the right. The sequence of movements of a comb-plate can be followed by moving along the row from right to left.

the dexioplectic pattern. The basic character of these two patterns is the sme, and only the dexioplectic pattern shown by the membranelles of *Stentor* will be described. The waves travel along the row of compound cilia around the edge of the peristome of the animal (Fig. 49), and the cilia beat outwards, roughly at right angles to the row. Each compound cilium is built of 60 to 75 cilia, being 3 cilia wide in the diaplectic direction (in *Stentor polymorphus*) and 20 to 25 cilia wide in the orthoplectic direction, except in the gullet region where fewer component cilia are present. The beating cilia do not touch each other because adjacent cilia beat in parallel planes, although the water movements that they create may cause some viscous interaction between adjacent cilia. The form of the individual waves was described above and shown in Fig. 45. The beating of each cilium is followed after an interval of about $\frac{1}{6}$ cycle by the beating of the next distal cilium to give the pattern of waves shown in Fig. 49. Diaplectic metachronal waves do not seem to show reversal of beat, but this apparent absence of reversal may be because it would have no functional value in the cases observed, and not that it is not possible.* In backward-swimming of

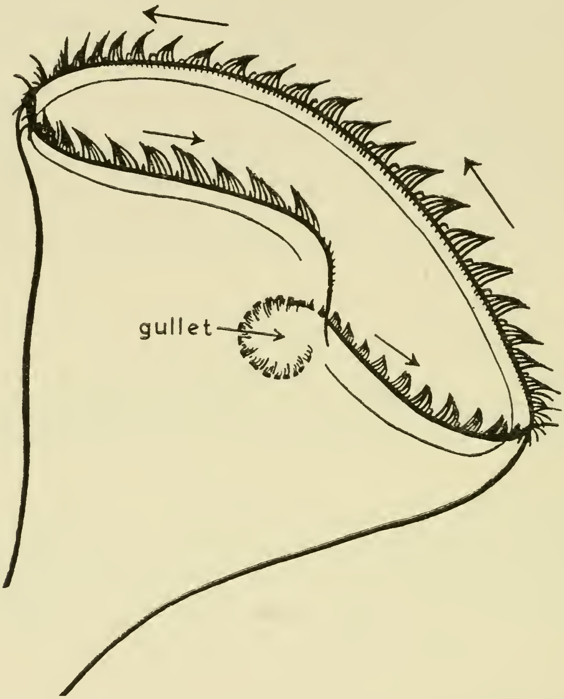


FIG. 49. Diagram of the peristome region of *Stentor* showing the arrangement of membranelles and the direction of metachronal wave transmission (from Sleigh, 1957).

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* Párducz (1961) has published photographs of *Didinium* fixed during both forward- and backward-swimming; these suggest that the metachronism is always dexioplectic, and that both the beat and the metachronal waves reverse.

Stentor, caused by the action of the body cilia, the membranelles are either quiescent or may waggle feebly without a co-ordinated beat.

The functional value of these different patterns of metachronism is rather different, as Knight-Jones (1954) has pointed out. Symplectic metachronism is probably the most primitive type; it does not make the best use of the movement of the cilia, for they are bunched together during the effective stroke. Diaplectic metachronism is especially suited for the creation of water currents in locomotion, feeding and respiration, and is probably not very easily adapted for the movement of larger particles or strings of mucous. Perhaps the fact that diaplectic metachronism is normally shown by cilia arranged in short orthoplectic rows reflects this. Antiplectic metachronism may also be used for creating water currents, but it is most commonly found in cilia whose function is the movement of particles or strings of mucus.

In both diaplectic and antiplectic metachronism the cilia have a greater freedom of movement than in the symplectic type, and are able to sweep through a large body of water quickly; they can be long and compound without interfering with the effective stroke of their neighbours, so that very large water currents can easily be created. The lack of interference in diaplectic types is fairly obvious, but in antiplectic metachronal waves the cilia must be spaced fairly widely to make full use of the beating activity, and the waves must travel sufficiently fast relative to the rate of beat that cilia in their recovery stroke do not get in the way of cilia which are in their effective stroke; this is particularly necessary with very large cilia like those of ctenophores. While a very large force can be exerted by these comb-plates of ctenophores, they must either be widely spaced, or lose efficiency by interference and reduced amplitude. The cilia of the rows along which diaplectic waves pass may be packed very tightly together and still retain a large amplitude of beat, and although it seems inconvenient or unnecessary for them to be too large, this may be because of their function in the cases so far examined.

Ciliated epithelia showing diaplectic metachronism are normally either in the form of narrow diaplectic bands with only a few cilia in each orthoplectic row, e.g. lateral cilia of *Mytilus* gill, or in the form of single diaplectic rows of (usually compound) liaic, e.g. the membranelles of *Stentor*, the velar cilia of nudibranch mollusc larvae, or the laterofrontal cilia of *Mytilus* gills. Narrow diaplectic bands are common on gill structures, feeding tentacles and swimming appendages or zones of the larvae and adults of many invertebrates including protochordates, as well as in some protozoans. They may form long bands such as those which run the length of the gill filaments of lamellibranchs, or they may form encircling rings around cylindrical gills, as in some polychaete worms (Knight-Jones, 1954). The greater specialization of antiplectic and diaplectic metachronism may be correlated with a more complex mechanism of metachronal co-ordination in these types, for the viscous interaction between beating cilia is normally much less here than in symplectic metachronal waves.

4. The Mechanism of Metachronism

The autonomy of beat of isolated cilia was stressed by Verworn (1889), for he had observed many times that cilia on cell fragments beat until they died, provided sufficient basal cytoplasm was present. He was concerned to discover how this autonomy of the cilia could be suppressed or controlled in order that the observed metachronal co-ordination should take place.

For many years there have been two widely supported theories concerning the mechanism of metachronal co-ordination. Thus, Engelmann (1868) believed that nerve-like impulses were responsible for co-ordination, and while some of Verworn's studies led him to believe that co-ordination was by mechanical action between ciliary shafts, he could not explain all of his observations on this basis (Verworn, 1891). The investigations carried out by Gray (1930) led him to consider these two possible co-ordination mechanisms, i.e. (1) a neuroid transmission mechanism in which cilia are excited by stimuli transmitted through the basal protoplasm, and (2) a mechanical interaction mechanism in which the movement of one cilium may influence the next by the viscous drag communicated through the surrounding water. He believed that the second was the more likely because the first could not be reconciled with the facts that (a) since cilia are autonomously active, the only stimuli that could control their beating were inhibitory ones, (b) the rate of wave transmission was so slow in comparison with the transmission of nerve impulses, and (c) meta-

TABLE 13. THE RATE OF TRANSMISSION OF METACHRONAL WAVES IN GROUPS OF CILIA SHOWING VARIOUS TYPES OF METACHRONISM

Species and type of cilia	Metachronal pattern	Metachronal wave velocity (μ/sec)	Cilia excited per sec	Temp. °C	Source reference
Opalina (body cilia)	Symplectic	100–200	*c.* 200[y]	10[z]	Okajima (1953)
Mytilus (lateral)	Laeoplectic	125		10[z]	Aiello (1960)
Mytilus (lateral)	Laeoplectic	308		23	Aiello (1960)
Mytilus (lateral)	Laeoplectic	169	*c.* 420	?	Lucas (1931a)
Mytilus (lateral)	Laeoplectic	160		15	Gray (1930)
Mytilus (laterofrontal)	?	60·3		?	Lucas (1931a)
Modiolus (lateral)	Laeoplectic	49		15	Lucas (1932b)
Stentor (membranelles)	Dexioplectic	657	173	18·5	Sleigh (1956b)
Paramecium (vestibular cil.)	Antiplectic	712	228	20	Sleigh (unpub.)
Pleurobrachia (comb-plate)[x]	Antiplectic	*c.* 7,500	*c.* 75	20	Sleigh (unpub.)

[z] —values measured at 10°C should be about doubled for comparison with measurements at 20°C or just below;
[x] —measurements made on detached comb-row;
[y] —using ciliary separation measurements from other studies.

chronal waves could start and stop at any part of an epithelium, and did not necessarily start at any particular pacemaker. It seems likely on more recent evidence that both of these hypothetical mechanisms suggested by Gray may occur naturally; Kitching (1961) has also hinted that this may be true.

It is evident from studies on *Stentor* (Sleigh, 1956a) that the fundamental variables of a group of metachronally co-ordinated cilia are the frequency and amplitude, which describe the beating activity, and the metachronal wave velocity which describes the co-ordination activity. Metachronal wavelength is probably not a fundamental variable of the system.

The rate of transmission of metachronal waves has been accurately measured by two methods. The more direct of these involves the use of a cine camera which exposes a known number of frames per second; the distance travelled by a wave in a known time can be measured from a film of the active cilia. The second method is somewhat more convenient in use; it involves the measurement of frequency with a stroboscope, and the simultaneous photographing of the metachronal waves, either using the stroboscopic illumination or else a separate flash-light. The wavelength of the metachronal waves may be measured from the photographs, and the product of wavelength and frequency is the metachronal wave velocity. Measurements made in these ways are included in Table 13, where it is seen that our information is rather limited.

Let us consider two of these examples of metachronism in detail, the slow symplectic metachronism of *Opalina* and the fast dexioplectic metachronism of the membranelles of *Stentor*, both of which are fairly well known. In *Opalina* the frequency and the wave velocity of the body cilia vary in direct proportion, so that variations in frequency along the length of the body are accompanied by changes in metachronal wave velocity (Okajima, 1953). In *Stentor*, small changes in the frequency are not necessarily accompanied by any changes in the metachronal wave velocity. If the viscosity of the medium is slightly increased, the frequency and metachronal wave velocity of *Opalina* cilia are reduced (Potter, 1960; Kitching, 1961), while in *Stentor* the frequency is reduced and the metachronal wave velocity remains unchanged. A further increase in the viscosity around *Stentor* membranelles results in a large *increase* in the metachronal wave velocity to well over 2 mm/sec in some cases.

When studying the movement of *Opalina* cilia, the observer gains the impression that their beating activity could be forced more or less into phase by the viscous interaction between cilia moving in the same direction, and that this interaction may act throughout the beat. This has been pointed out by Kitching (1961), who also believes that the observations made on *Opalina* cilia are those that one would expect if the cilia were co-ordinated mechanically by viscous interaction. Thus, when the beat is slowed down, the wave velocity is also reduced. Where the frequency is reduced in viscous media, the amplitude of beat is also reduced, so that the wave velocity is reduced by more than the frequency, and the wavelength is decreased. One surprising feature of this metachronal transmission in *Opalina* is that the rate of transmission is of the same order whatever its direction, e.g. the rate of propagation of the waves across the ciliary rows may be about twice that along the rows, while the separation of the cilia is some six times as great; the faster transmission across the body probably results because the cilia beat faster in this direction, and not because of the greater separation of the cilia. It appears that the metachronal wave velocity is directly controlled by the rate of beat of the cilia, and that even when they are at their maximum separation they will still be close enough to excite each other mechanically.

The undulatory waves of various organelles are known to be forced into phase, e.g. Gray (1928) reported this from swimming spirochaetes and it is well known in the tails of spermatozoa; it has been calculated by Taylor (1951) that it is much easier for such tails to beat in unison. Examination of a metachronal wave of *Opalina* (Fig. 47 and Pl. XVIIIb) shows that a similar effect could be operating here, where, although each cilium of the metachronal wave lags or leads on its neighbours in the plane of beat, the cilia at right angles to this plane beat synchronously. It may be a little difficult to visualise why mechanical interaction should be able to give synchrony at right angles to the beat rather than metachrony, when one might expect the viscous or other mechanical interaction to be less at right angles to the beat. After disturbance of the animal, waves appear simultaneously over the whole surface of the animal, and soon settle to the usual pattern by coalescence and elimination. It looks as if, once the cilia have started to beat in a particular direction, their activity is modified so as to cause the minimum of mutual interference (the direction of beat is controlled independently, see p. 193). The first cilium to beat may influence those around it, but the overall activity must depend on the average speed with which the cilia can beat, for faster cilia could here be slowed down by others. Cuts across the animal do not modify the passage of waves in either part of the animal, so that the frequency of beat seems to be determined by the cilia forming the waves. It is interesting here that the longer cilia towards the posterior end of the body normally beat more slowly than the anterior ones, but some waves coalesce so that the waves are longer posteriorly. When two waves coalesce, however, the wavelength does not remain at twice the normal length, for there is a rapid mutual adjustment between adjacent waves so that they are all of about the same length. All observations seem to point to some type of mechanical co-ordination process, but further observations on this type of metachronism are urgently required.

The metachronism of the membranelles of *Stentor* seems to be of a quite different type at normal viscosities, since the frequency and metachronal wave velocity are differently affected by temperature, small viscosity changes, magnesium chloride and digitoxin (Sleigh, 1956a). Two of these factors which affect the mechanical activity of the cilia do not influence the metachronal co-ordination at all. This metachronism has the appearance of an internal or neuroid conduction process passing through the internal cytoplasm or the surface layer of the cell and triggering off the ciliary contractions.

It appears that there are two ways in which such a system might operate (Sleigh, 1957). Firstly, a nerve-like impulse might be conducted along the length of the row of cilia in such a way that the cilia are stimulated to beat as the impulses pass, but do not participate in the transmission of the metachronal impulses. Secondly, there could be a step-by-step or " stepwise " process passing across one interciliary space at a time, in which the excitation of one cilium results in the production of a conducted impulse responsible for the stimulation of the next cilium in the row, as well as triggering the contraction of the first cilium. One drawback to the first idea, which was pointed out by Gray (1930), is that the rate of transmission is very slow in comparison with other conduction systems, so that at least some other mechanism must be involved. The second suggested mechanism implies that two parts are involved in the wave transmission, an intracellular excitation process and an intercellular conduction process, and the actual conduction could be of the speed found in nerve if the excitation takes up a large part of the total transmission time. It is interesting that the wave velocity of the membranelles of *Stentor* is increased by digitoxin, which is believed to lower the threshold of excitability in heart muscle.

Two observations throw some light on this, and both of them point towards the stepwise theory of transmission. When the row of membranelles along the edge of the peristome of *Stentor* is cut across, the frequency of the distal region almost always changes (by up to 20 per cent) to a new level at which it remains until the cut heals, which is usually within an hour for a reasonably small cut, and then the frequency returns to the previous level. The frequency change is accompanied by a change in wavelength so that the wave velocity in the distal region remains the same as in the proximal region throughout the experiment. It appears that the wave conduction is dependent on the cytoplasm through which the waves are conducted, and again the transmission is independent of the frequency. The frequency is the same throughout any row of membranelles that are in physical continuity, but as soon as there is a break in the row, there is a change in the frequency from the break to the end of the row. In these cutting experiments the frequency in the distal region is usually lower, but occasionally higher, than in the proximal region. Restoration of the continuity of waves across the cut and uniform frequency throughout the row normally occurred as soon as the cut edges came together, even when the cut edges did not meet at exactly the same level; because of this last observation it is unlikely that any specialized conducting structures, such as fibres, are concerned with the metachronal transmission.

Membranelles of the gullet region of the peristomial field of *Stentor* are interesting in that they are smaller and show a shorter metachronal wavelength than those in the main part of the row;

since the frequency is the same throughout, the wave velocity must be lower in this region of the row. Measurements of the wave velocity taken in the gullet region and in the main part of the row are shown in Table 14. It is also interesting that the cilia in this region of the row are closer together than in the main part of the row, and it was found that the number of cilia in each wave and the number of cilia stimulated per second is constant throughout the row (Table 14). Hence it appears that the conduction depends on the number of cilia involved in the transmission, rather than on the linear distance. These results would not be out of place in a stepwise conduction system where the excitation takes the main part of the total conduction time. Although it is just conceivable that the cilia of the gullet region could give a slower conduction by reduction of mechanical interaction, because they are smaller than the large cilia, the fact that the frequency and wave velocity are independent makes this very unlikely indeed.

TABLE 14. THE VALUES OF VARIOUS PARAMETERS OF CILIARY ACTIVITY IN THE GULLET REGION OF THE MEMBRANELLE ROW OF *Stentor*, COMPARED WITH THOSE IN THE MORE DISTAL REGION OF THE SAME ROW OF CILIA

	Gullet region	Distal region
Wavelength	15·9 μ	24·2 μ
Frequency	27·4 beats/sec	27·4 beats/sec
Wave velocity	436 μ/sec	613 μ/sec
Interciliary distance	2·68 μ	4·06 μ
Cilia in each wave	5·95	5·97
Cilia stimulated per second	163	163·5

The behaviour of regions of the membranelle row isolated by cuts led to the pacemaker theory (Sleigh, 1957). Cilia which are isolated from all others normally show an autonomous beating activity, and yet, in an epithelium or in a row with others, they may be controlled and made to beat in a co-ordinated rhythm. Such control could be exerted if the co-ordinating impulse was to arrive before the cilium was ready to show a spontaneous beat. If each cilium shows a spontaneous build-up of excitation to a threshold, and the conducted impulse accelerates this build-up, then the conducted impulses could control the rate of beating of the cilia. It is noteworthy that in any row of *Stentor* membranelles the frequency is normally the same throughout the row from the first cilium, so that it appears that the first cilium of any row initiates the rhythm for that row, i.e. it is the pacemaker. The pacemaker cilium communicates its rhythm to all the cilia that follow it in the row, but in all cases where this communication is prevented, another cilium takes over the pacemaker function. The first active cilium beyond the cut in the experiments quoted above was the pacemaker for the cilia in the distal region, and although it normally shows a frequency lower than it did before the cut was made, a higher frequency, which could have resulted from a local injury to the cilia, was occasionally measured. Occasionally the metachronal transmission from the gullet region to the distal end of the row may be disrupted by natural means, e.g. when a large object is carried into the gullet or when the edge of the peristome is caught up by some object in the environment. When either of these things has occurred, the frequency in the distal region has always been found to be reduced. It is also interesting that the transmission may be prevented by stoppage of the beating of the cilia in these cases, which provides additional evidence for the stepwise mechanism proposed above.

The action of the pacemaker and the postulated transmission mechanism in *Stentor* are illustrated in Fig. 50, in which a pacemaker cilium is shown passing rhythmic transmitted impulses to two cilia which follow it. The rate of beat of the pacemaker depends on factors discussed in Chapter IV (p. 167). It is interesting that this idea of a pacemaker is directly comparable with that found in the heart of vertebrates; the chambers of the frog heart have intrinsic rhythms decreasing in the order sinus, atria, ventricle and bulbus cordis, yet in the whole heart all parts beat with the rhythm originated by the sinus.

Although this system of metachronal transmission appears to be used under most experimental conditions by these membranelles, the co-ordination is modified in media of high viscosity (p. 84). In a very viscous medium the metachronal wave velocity is increased, apparently because the excitation of the cilia is caused by viscous drag communicated through the medium.

The higher the viscosity, the faster the co-ordination, apparently because the viscous interaction is increased. Since the amplitude of beat is progressively decreased with increase of viscosity, a point is eventually reached where the viscous interaction is no longer effective and the co-ordination breaks down. It seems probable that this explanation is the correct one, for slight fluctuations of the frequency occur from time to time, and, while slight decreases of frequency in the normal medium are accompanied by small increases in wavelength (i.e. wave velocity constant), similar decreases of frequency in a very viscous medium are accompanied by a decrease in wavelength.

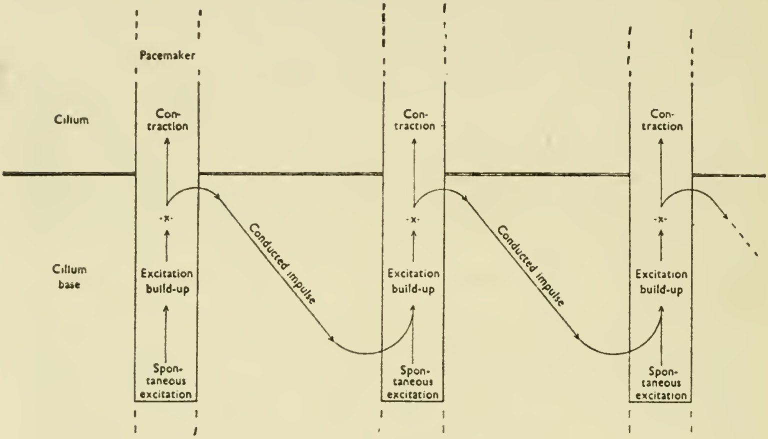

FIG. 50. A diagrammatic representation of the theory of metachronal co-ordination proposed in the text. The spontaneous build-up of excitation in the pacemaker cilium determines the frequency of beat of that cilium, and other cilia beat at constant intervals after this as a result of the conducted impulses (from Sleigh, 1957).

Considerable evidence concerning the metachronism of the lateral cilia of *Mytilus* and related lamellibranch molluscs has been accumulated, particularly by Gray (1930), Lucas (1932a, b), Aiello (1960) and Gosselin and associates (1958, 1961). Throughout these studies it has been the common experience that any factor which affects the frequency also changes the wave velocity. In drugs like veratrine or serotonin for example the frequency may be increased by as much as 100 per cent, but the wavelength remains practically unchanged. Similarly, Gray and Lucas found that the wavelength remained unchanged when the frequency was changed by altering the temperature, although Aiello found a small increase in wavelength when the temperature was raised. An important observation was made by Gosselin (1958), who found that an increase in viscosity did not change the frequency of beat, but reduced the amplitude of beat and increased the metachronal wave velocity. All of these observations could be adequately explained on the basis that these cilia are co-ordinated mechanically through the medium and do not depend on an internal conduction mechanism.

The metachronism of the lateral gill cilia is laeoplectic (Knight-Jones, 1954; Aiello, 1960). The metachronal wave-front is at right angles to the line of transmission of the waves, yet the rows of ciliary basal bodies are at an angle of about 120° to the gill filament axis. Aiello found that the cilia beat roughly along these rows, i.e. obliquely, while Lucas found that the cilia beat directly across the axis of the gill filament. If the cilia are very close together and beat slightly obliquely, then mechanical co-ordination could take place through a combination of viscous drag and the cilia striking the cilia obliquely ahead along the axis of wave transmission. If the beat is exactly transverse, only the viscous drag can be effective. The metachronism of these cilia is certainly more irregular than that of *Stentor*, and varies from place to place along the row as one might expect if the metachronal co-ordination is mechanical rather than neuroid. Lucas thought that an internal transmission mechanism was likely because waves of the normal frequency and size appeared on the distal side of quiescent regions, but this could equally be the result of the action of a pacemaker distal to the quiescent cilia which communicated its rhythm mechanically to the distal cilia. Further work on these cilia, with a view to establishing the possibility of mechanical co-ordination, is eagerly awaited.

It is evident that many groups of metachronally co-ordinated cilia will have to be studied in considerable detail before we can see whether any general pattern exists, and that any further suggestions at this time would be pure guesswork. However, it is probably reasonable to speculate that where cilia show diaplectic or antiplectic metachronism in which the cilia are widely spaced along the line of metachronal transmission, and the transmission is rapid, then co-ordination is probably internal and of the neuroid type. In addition to *Stentor* membranelles, it seems that we can here include the metachronism of ctenophore comb-plates, for Verworn (1891) found that metachronal waves were transmitted past a plate whose movement was restrained so that it could not touch any other plate. On the other hand, it seems likely that cilia which are close together and show a slow metachronal co-ordination depend on mechanical transmission through the medium, especially where the metachronism is symplectic. Experimentation with viscous media is a valuable tool in these investigations, but it must be remembered that different results must be expected from different metachronal patterns, and it is in those cases where a change in viscosity does not affect the metachronal transmission that an internal transmission mechanism is likely.

5. Some Thoughts on the Origin and Development of Metachronism

Small groups of flagella may beat synchronously because of viscous interaction between their undulating shafts. Undulating flagella may occur in large numbers over considerable surface areas, where they are arranged so that they lie roughly parallel to the surface which bears them, as in the trichonymphid flagellates. It is not surprising that, in these cases, the same viscous interaction which produces synchrony between the shafts which lie side by side, also produces metachronism of the flagellar beat in that flagella further back on the body start to beat later than those further forward on the body (see Fig. 51). These flagella are more or less fixed in their orientation on the body, and can only produce forward movement; changes of direction are caused by the " steering " action of the anterior end of the body.

The cilia of *Opalina* are shorter than the flagella of trichonymphids, yet the appearance of waves of beating organelles is very similar in the two types, as can be seen by comparison of Fig. 51 with Fig. 47. Waves of contraction passing along the shafts of cilia must cause viscous interaction with those that lie underneath, and which have an origin further back on the body surface. These cilia have no fixed orientation of beat, and, by changes in the direction of beat, the direction of metachronal wave transmission may also be changed (see p. 193). Changes in direction of movement that are caused by this means may be much more difficult in a longer organelle, and, although the viscous interaction is reduced in the shorter organelles, the cilia of *Opalina* may have been shortened in order that the direction of movement of this fairly rigid body can be changed. It is not known, at present, how widespread the symplectic pattern of ciliary metachronism may be, and no other examples of this pattern are well enough known for any comparison to be made with *Opalina* cilia.

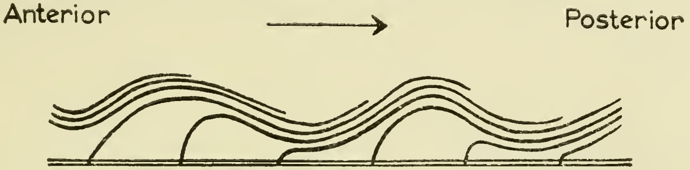

FIG. 51. Diagram of flagella of the posterior region of a trichonymphid flagellate. The flagella may be forced to beat synchronously by viscous interaction, and flagella further back on the body will start their beat later than those further forward, i.e. they show metachronism.

The next stage in the development of metachronism is more difficult to visualize, but it seems possible that the antiplectic pattern preceded the diaplectic patterns, since the former is more nearly related to the primitive symplectic type. If the cilia of *Opalina* were shorter and spaced further apart, so that they could move through a large angle in the effective stroke without interfering with other cilia, then an antiplectic pattern could easily be produced by a small adjustment of excitation intervals (see p. 158). Thus, a change from a phase difference of about one-sixth in the approximately symmetrical beat of *Opalina* cilia to a much smaller phase difference of say one-twentieth in the asymmetrical beat of the vestibular cilia of *Paramecium* (Fig. 52), could result in a change from the symplectic to the antiplectic patterns. Although antiplectic metachronism may require co-ordination through the basal protoplasm, viscous interaction may be present in the recovery phase in some cases (e.g. Fig. 52), and this may be sufficient to maintain the rhythm. Where such metachronism is found over large areas of ciliated surface, e.g. on the pharyngeal epithelium of the frog and probably the vestibular ciliature of *Paramecium*, it is important that cilia at right angles to the direction of travel of the metachronal waves beat synchronously, or nearly so, presumably under the influence of mechanical interaction.

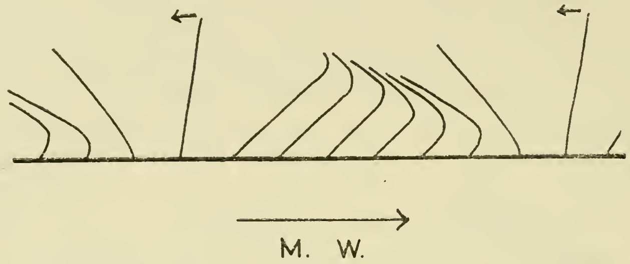

FIG. 52. Diagram of a metachronal wave of the vestibular cilia of *Paramecium*. The metachronal wave is moving towards the right, and the effective stroke is towards the left. There is a possibility of mechanical interaction between the cilia in the recovery stroke, but this is less likely in the effective stroke.

Narrow tracts of such ciliated surfaces could give either antiplectic bands of cilia, if the tract is composed of short diaplectic rows and long orthoplectic rows, e.g. the frontal cilia of *Mytilus* gill, or diaplectic bands of cilia with short orthoplectic rows and long diaplectic rows, e.g. lateral cilia of *Mytilus* gill. In both cases the metachronal waves could originally have been mediated by mechanical interaction, and could have been modified for co-ordination by neuroid impulses by some mechanism like the stepwise conduction suggested earlier.

The ideas expressed here are almost entirely speculative, but may form some basis for experimentation.

6. Ciliary Reversal

Change in the beat direction of cilia is known to occur in the ciliated protozoa and *Opalina*, and many experiments have been carried out to discover the conditions under which it takes place.

In metazoa, anthozoan coelenterates (e.g. *Metridium*, Parker, 1928), ctenophores (e.g. *Mnemiopsis*, Coonfield, 1934) and nemertines (Friedrich, 1933) have been found to show temporary reversal of beating activity, but information from other groups is very limited. There have been reports that the beat of some cilia may be permanently reversed for the life of the animal, e.g. some frontal cilia of *Mytilus* gills (Atkins, 1930), but this may result from a secondary development of special cilia, and comes in a different category.

It appears that reversal of beat in *Metridium* is a necessary part of the normal behaviour of the animal, and is required in feeding. Parker (1928) found that in *Metridium* the cilia on the " lips " around the mouth normally beat outward, and would carry away a scrap of filter paper soaked in seawater, but would reverse and carry into the mouth similar scraps of filter paper soaked in glycogen. Similarly, mussel extract causes reversal of the beat of these cilia (Parker and Marks, 1928). Reversal of beat in *Mnemiopsis* and some other ctenophores seems to be associated with the body movements necessary to bring the animal into such a position that it can swallow the food caught by the tentacles; these movements must require a high degree of control and co-ordination of the cilia.

Reversed beating of the cilia of *Paramecium* and *Opalina* is also a normal part of everyday life for these organisms. In fact, in both types the cilia may beat in almost any plane (Okajima, 1953; Parducz, 1954), and the animal is not restricted to movement in the forward direction only, but can reverse and turn according to the direction of beat. It has already been mentioned that electric current (p. 90), some ionic changes in the medium (p. 98) and membrane deformations (p. 95) will cause ciliary reversal, and in life a wide variety of stimuli such as contact with obstacles in the environment will cause a reversal of beat for a time before forward beating is resumed.

From many of these studies it appears that reversal of beating is accompanied by reversal of the metachronal wave. Some different patterns of metachronal waves in *Opalina* and their movements over the " dorsal " and " ventral " surfaces of the body at different stages in the change of beat direction are shown in Fig. 53. These patterns are commonly observed in the normal swimming of *Opalina*, and it appears that the wave pattern changes virtually instantaneously over the whole body. Increasing excitation causes a progressive change in the wave pattern, as shown in stages I to VI in Fig. 53, until the waves travel forwards from the posterior end. The change in direction of beat and the increase of excitation were found by Kinosita (1954) to be accompanied by an increased depolarization of the surface membrane of the animal.

In a recent review of this subject, Kitching (1961) has suggested that the progressive depolarization of the surface membrane changes the site of pacemaker activity from the anterior end along the " right " side of the body in transverse beating to the posterior end in reversed beating. Indeed, Okajima (1953) implies that the frequency of beat increases with increased excitation, and this would allow different pacemakers to control the beating activity of the cilia by their higher rate of stimulation. However, the direction of beat of cilia on a small area of the body surface is changed by a localized depolarization under conditions where the main pacemakers of the body (if these exist) are not affected at all. This leads to the alternative suggestion that the depolarization of the surface membrane affects every cilium, not just the pacemaker, so that the direction of beat of every cilium is changed by a greater or lesser extent according to the amount of

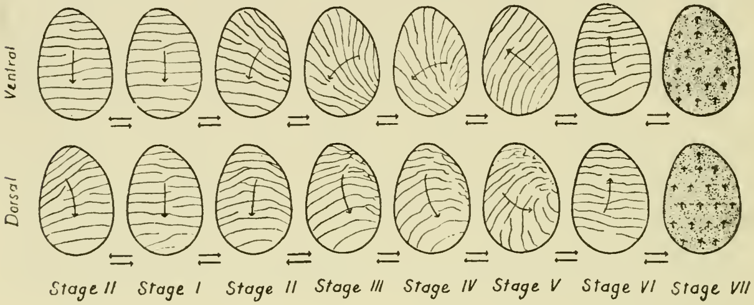

FIG. 53. Metachronal wave patterns at various stages in the reversal of ciliary beat in *Opalina*. The arrows on the figures indicate the direction of metachronal wave movement. The wave patterns on the ventral surface were observed through the transparent protoplasm (from Okajima, 1953).

depolarization. Naitoh (1958) found that the membrane showed a difference in sensitivity at different parts of the body surface; this was at least partly attributable to differences in membrane resistance, so that the same amount of depolarization may give different degrees of change in beat direction at different parts of the body surface as in Fig. 53 (especially Stage IV ventral). This depends on the assumption that the depolarization spreads over the whole cell without appreciable decrement. It is interesting that the direction of beat changes apparently simultaneously over the whole body of *Opalina*, and further, Tartar (personal communication) has found that where several stentors have been grafted together, body cilia over the whole surface of the joined mass reverse simultaneously. Incidentally, the beat of the membranelles is inhibited at the same instant. A rapid spread of electrical depolarization over the surface of these protozoa is thus held to cause a change in the direction of beat, and this would explain why the reversal response affects the whole surface of an animal like *Paramecium* even when parts of the body have been almost isolated by cuts.

It is also interesting to speculate on the mechanism of reversal in these protozoa. As Kitching suggests, there may be a parallel between the relationship of depolarization to ciliary reversal and the relationship of depolarization to muscle contraction, and it is tempting to suggest that in both cases ions, particularly potassium ions, are intimately involved. The experimental production of reversal involves treatments which either upset the balance between monovalent and multivalent cations, or upset the stability of the membrane electrically or mechanically. Either treatment could permit a change in the pattern of ionic movements across the membrane. While an increased concentration of monovalent cations outside the body will induce reversal and divalent ions will not, the excitability for this reversal is reduced in the absence of calcium and increased in calcium-rich media (Kinosita, 1954; Okajima, 1954b). The fact that the injection of calcium precipitants induces prolonged reversal of beat in *Opalina* (Ueda, 1956), may be another result of ionic unbalance or may have a more specific effect in that reduction of internal calcium concentration induces reversal. It seems possible, then, that the membrane depolarization involved in reversal permits ionic movements which interfere with the normal excitation of the cilia in such a way that a different fibril of the cilium is caused to contract first, and the direction of beat is changed. Different fibrils of the cilium may contract first under different conditions of excitation, and it is interesting that the change in wave pattern with depolarization always takes place in the same direction.

Little evidence is available about the reversal of ciliary beating in the metazoa, but in the well-known cases it is evident that the reversed ciliary beat is started by a contraction in the fibrils on the opposite side of the cilium from those which start the normal beat, and a mechanism similar to that suggested for protozoa could be involved.

Reversed beating has been reported from cilia whose metachronism is symplectic (*Opalina*) or antiplectic (*Mnemiopsis*), and in both cases it is known that the direction of metachronal transmission is also reversed.* Taylor (1920) reported that the membranelles of *Euplotes* showed reversal; these compound cilia show diaplectic metachronism, but it is not known quite how the beat changes in reversal or whether the transmission of the metachronal wave is also reversed.

It is interesting that the cilia forming the cirri of hypotrichs like *Euplotes* are in isolated groups and cannot therefore be metachronally co-ordinated, but yet these cirri are capable of beating in at least two directions in co-ordination with other cirri (Taylor, 1920). The cirri are very often quiescent, but may be excited to beat forwards or backwards, and perhaps also in other planes. Excitation involves the passing of two items of information, the stimulus to beat and some indication of the beat direction. Taylor showed that the " neuromotor " fibres of *Euplotes* were important in co-ordination in that the beat of the various cirri was synchronized when the fibres were intact, but not when the fibres were cut. As a pure speculation, it is possible that the fibres may be used to excite the beat of the cirri, while the electrical depolarization of the surface membrane used in the reversal of beat in other ciliates may be used to determine the direction in which the cirri beat. It is interesting that reversed beating of cirri may be accompanied by the inhibition of movement of the membranelles, and that the membranelles of *Stentor* cease to beat when the beat of the somatic cilia is reversed. The alternative suggestion that cirri are normally inactive because of inhibition is also a possibility, for Taylor found that cirri isolated with small masses of protoplasm are continually active, but there is no proof in this finding because the surface membrane and protoplasm may have lost their normal constitution. It is worth mentioning again that the co-ordination of the cirri is not metachronal co-ordination, although the excitation of the beat of cirri may employ the mechanism used in at least some types of metachronism.

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* See footnote on p. 178

7. Inhibition and Activation

Almost all cilia and flagella seem to be under the control of the organism, and such control may involve activation, inhibition and sometimes reversal. Examples of these types of control were quoted in Chapter III; the interesting example of the control of ciliary beat in *Euplotes* has just been mentioned, and here as in many other cases it is difficult to be certain whether activation or inhibition is involved.

The stoppage of cilia that are normally continuously active is almost certain to be a genuine case of inhibition, and it is interesting that inhibition of the movement of membranelles of *Stentor* occurs at the same instant as reversal of beat of the body cilia. It may be possible, therefore, that complete inhibition of beat may be controlled by the same mechanism as reversal, and that the membranelles respond to depolarization by ceasing their beat, while the body cilia reverse. Perhaps the thigmotactic inhibition of *Paramecium* similarly involves a depolarization or hyperpolarization of the membrane, and may be an extension of the change that causes reversal of beat.

Inhibition of ciliary activity in metazoa is believed to result from nervous activity, in gill cilia of *Ostrea* and locomotory cilia of molluscan veliger larvae for example (see pp. 121-124). In no case is the mechanism known.

Activation of cilia by the stimulation of cilia that are normally quiescent or increase in the activity of slowly beating cilia is believed to occur in the cirri of hypotrichs and also in metazoans, e.g. the lateral cilia of *Mytilus* gill (p. 122). This phenomenon is again likely to bear a close relationship to the normal excitation of cilia, and even if the mediator between the nerve and the cilium of *Mytilus* is serotonin, the mechanism of its action on the cilium is obscure. Although speculation here is certainly premature, ions may well be concerned in activation as in so many biological phenomena; we badly need to know more about the effects of ions on ciliary activity.

8. Root-fibre Systems and Co-ordination of Ciliary Beat

The metachronal waves of the body cilia of organisms like *Opalina* and *Paramecium* may be transmitted in directions which do not correspond to the rows of cilia. It is now well known that in both of these organisms there is a system of more or less longitudinal fibres which connect with the basal bodies of the cilia, although the fibres are rather different in the two examples. These fibre systems have often been held to be responsible for the transmission of the metachronal impulses, but in view of the wide variety of wave patterns this seems unlikely (Pitelka, 1956; Párducz, 1958b). In *Opalina* at least, a reasonable explanation of metachronal co-ordination has been given without reference to such fibril systems. These fibrils seem more likely to be concerned in the " morphogenetic " relationships between the ciliary bases, and perhaps also serve a physical function in the anchorage of cilia and the preservation of pellicular shape, especially where there are special intuckings of the body surface. In *Stentor* the fibres connected to the bases of the somatic cilia are believed to be contractile.

The fibrils which connect more specialized cilia, such as the cirri and membranelles of *Euplotes* and the membranelles of *Stentor*, deserve a little more detailed consideration. Severance of the fibres that interconnect the cirri of *Euplotes* is known to disrupt their normal co-ordination (Taylor, 1920). It is interesting that these " neuromotor " fibres are built up of many tubular fibrils of a similar size to those which compose the connecting fibres between the somatic cilia of *Stentor*, although they are not lined up in the same way. Kitching (1961) has tentatively suggested that the neuromotor fibrils of *Euplotes* might function by the conduction of a propagated contraction, It would be interesting if Taylor's very valuable experiments could be repeated and extended, although it is difficult to analyse the movements of such a mobile animal.

The fibrous connections between the membranelles of *Stentor* were described on p. 64. If this row is cut across, the metachronal co-ordination of the membranelles is interrupted at the cut, but the waves flow smoothly across the position of the cut as soon as it heals, even if the two edges of the cut are not level. It seems unlikely that a cut fibre could heal up this quickly. Also, it has been noticed that the co-ordination may break down without any cutting of the fibre. These fibrous connexions can hardly be regarded as conductors on the basis of this evidence.

The presence of such organized fibrous connexions between the cilia of metazoans has not been recorded, although Fawcett and Porter (1954) reported knobs on the roots of cilia that project towards the adjacent cilium in the epithelium, and Gibbons (1961b) has found fine roots attached to these knobs. There is no evidence that any such fibre systems are concerned in metachronal co-ordination, although the crossed rootlets between cilia of the two rows of cilia that compose the laterofrontal cilia of *Mytilus* gill filaments may help to ensure a synchronous beat. It is interesting that prominent rootlets of some sensory cilia may be responsible for the conduction of the received impulse away from the cilium (e.g. in the locust scolopale cilium), and it may be equally possible for rootlets of this type to conduct impulses to the cilium. The relationship between the nerve endings and the roots and bases of the velar cilia of the nudibranch larvae described by Carter (1926) deserve further examination in the light of this idea, for these cilia are known from Carter's work to be subject to inhibition.

9. Summary

When single flagellar organelles are present on motile bodies, the flagellar shaft is usually modified in some way to increase its efficiency. Similarly, where cilia are required to exert larger forces, they tend to be compounded together, with the result that either the rate of beat or the volume of water swept through (or both) may be increased. The beat of many separate flagella or cilia present on the surface of a cell may be used most effectively if there is some form of co-ordination. Thus, the beating of the flagella of a small group is usually synchronous, for the waves of bending of the flagella are apparently aligned by viscous interaction. Cilia or flagella may clothe large areas of surface, and here synchrony of all of the organelles is not found, for, while cilia in one line on the surface tend to beat synchronously, those in the line at right angles tend to beat one after the other, metachronally.

Metachronal patterns have been classified according to the relationship between the direction of movement of the metachronal waves and the direction of the effective stroke of the ciliary beat. The following four groups have been named : symplectic antiplectic, dexioplectic and laeoplectic. The form of metachronal waves on the ciliated surfaces showing several of these patterns is well known, but they do not all appear to use the same mechanism of metachronal transmission. Thus, in *Opalina*, the transmission appears to be mediated through the viscous interaction between the shafts of the moving cilia, while in the membranelles of *Stentor* a more refined internal transmission mechanism seems to be involved. Evidence is available to suggest that this transmission moves step-by-step along the row of cilia, and involves two stages in each step : an interciliary conduction process and an intraciliary excitation process. In both types of metachronal co-ordination a ciliary pacemaker could control the rate of beat of all the cilia that beat after it. Under certain conditions the membranelles of *Stentor* may be co-ordinated to form metachronal waves under the influence of viscous interaction.

The beating of cilia and flagella usually seems to be under some form of control by the organism. In flagella, the effective movement of water relative to the organelle is restricted to one direction—from flagellar base to tip—and the only effective form of control is by activation or inhibition. The beat direction of many cilia is also fixed and they are controlled in the same way. In other cilia, however, a mechanism is present for changing the effective direction of the beat, so that a further means of control of ciliary action is available. The reversal of ciliary beating and metachronal waves seems to be closely connected with electrical changes; these may in nature result from ionic movements. There is no sound evidence to show that metachronism, or any other form of ciliary co-ordination for that matter, involves any form of fibrillar conduction.

APPENDIX

Some References to the Occurrence and Functions of Cilia and Flagella

General reviews on ciliary organelles :
- Gray (1928), von Buddenbrock (1961), Rivera (1962)—mainly physiology;
- Fauré-Fremiet (1961), Fawcett (1961)—mainly anatomy.

Cilia and flagella of protozoa :
- Calkins and Summers (1941)—general, physiology, etc.;
- Corliss (1961)—extensive bibliography of work on ciliated protozoa;
- Mackinnon and Hawes (1961)—general textbook on protozoa;
- Pitelka (1962)—ultrastructure of protozoa;
- Tartar (1961)—monograph on *Stentor*;
- Wichterman (1953)—monograph on *Paramecium*.

Cilia and flagella of metazoa :
- Atkins (1932, 1955, 1956)—ciliary feeding in ectoprocts, entoprocts and brachiopods;
- Chu (1942)—circulation of cerebrospinal fluid of the frog;
- Fjerdingstad (1961)—feeding in sponges;
- John (1933)—cilia in chaetognaths;
- Knight-Jones (1954)—metachronism of cilia of metazoa;
- Lucas (1932c)—distribution and functions of cilia in vertebrates (especially man);
- Meyer (1927, 1929, 1930)—ciliary circulation of coelomic fluid of invertebrates;
- Morton (1960)—functions of the gut in ciliary feeders;
- Orton (1913, 1914, 1922)—ciliary feeding in the amphioxus, ascidians, *Solenomya*, brachiopods, some polychaetes and *Aurelia*;
- Parry (1944)—cilia in the gut of chaetognaths;
- Purchon (1955)—structure and function in some rock-boring lamellibranchs;
- Slifer (1961)—insect sense organs;
- Wolken (1961)—photoreceptor structures;
- Yonge (1928, 1930)—feeding in corals and other invertebrates.

Some of these authors, and many others who have written on various specific topics, are referred to in the text. Further references to the occurrence and functioning of cilia and flagella in particular groups of animals may be found in the textbooks of Hyman (1939-59) and Grassé (1948-61).

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PLATES

I - XVIII

Plate I. Electron micrograph showing transverse sections of the flagella and basal bodies of *Pseudotrichonympha*.

The flagellar shaft structure is seen at the top, where several flagella are aligned in grooves, with connexions between some peripheral fibrils and granules on the inside of the walls of the groove. Sections lower down on the plate show the structure of basal bodies at successively deeper levels. Note especially: the distal end of C subfibrils and traces of the basal plate (at upper left), the three central cylinders (middle), and the connexions between the fibril triplets both through the hub and spokes and through A–C connexions (lower middle). From Gibbons and Grimstone (1960). × 95,000.

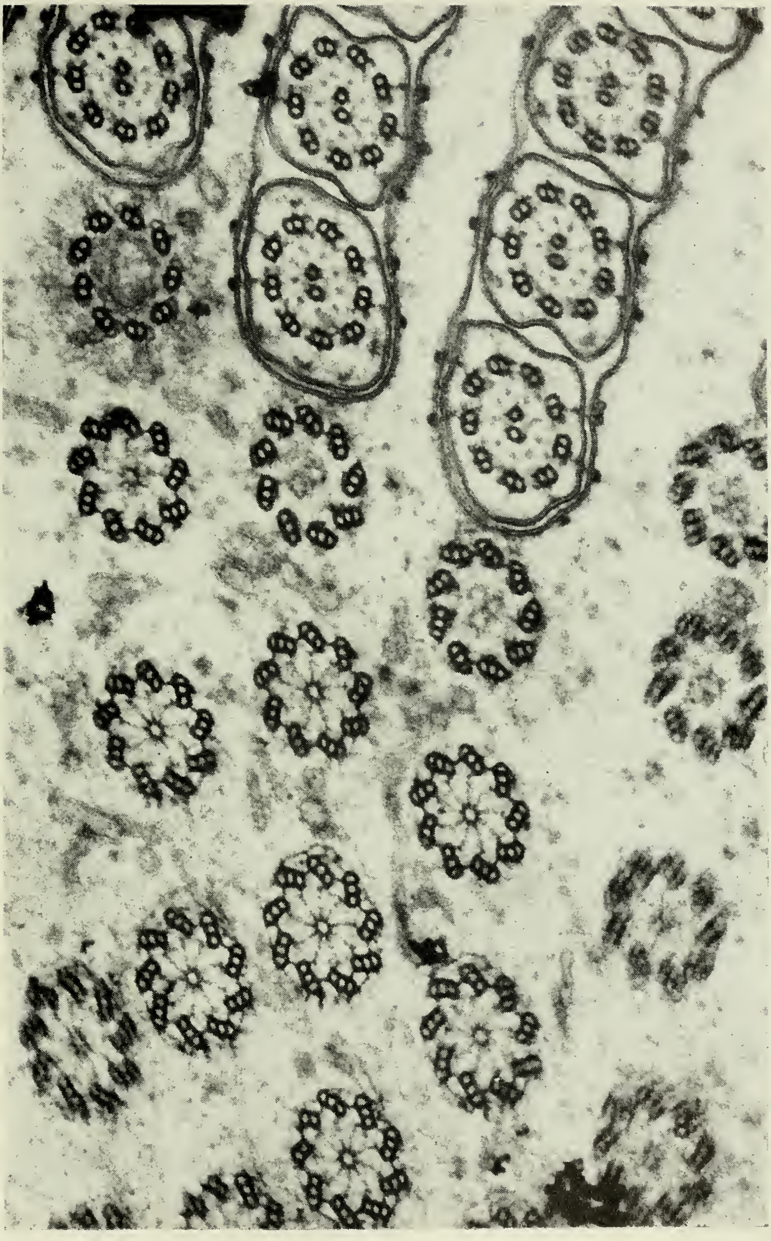

Plate II. Electron micrographs of sections of *Anodonta* cilia and their bases.

(*a*) Longitudinal section through the basal region of a frontal cilium. The section is perpendicular to the plane of the central fibrils and passes through the basal foot on the left. The structure of the transition region and basal body is illustrated in Fig. 7. Note that the main part of the striated root originates on the right, on the side opposite to the basal foot. × 47,000.

(*b*) Transverse section through ciliary basal bodies of a frontal cell. The basal feet of all the basal bodies project in the same direction (towards the effective stroke of the beat). Tubular fibrils run among the basal bodies and some connect with the basal feet. × 53,000.

(*c*) Longitudinal section of a short length of a frontal cilium. The two central fibrils may be seen, and some radial links and spiral striations of the central region are visible in places. × 84,000.

(*d*) Transverse section through the double row of cilia on a laterofrontal cell. The cilia beat towards the right in the effective stroke. Note that all have the same orientation with respect to central fibrils, peripheral fibrils and mid-filaments. × 34,000.

All micrographs from Gibbons (1961b).

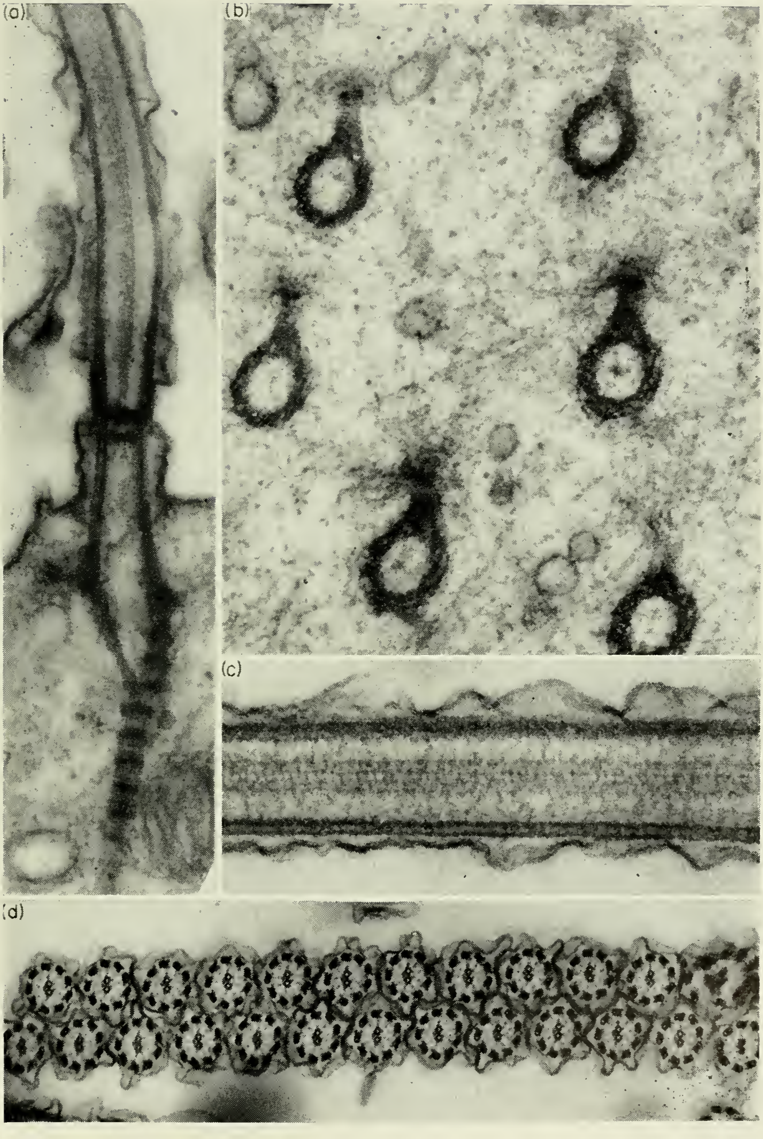
(a)
(b)
(c)
(d)

PLATE III. Electron micrographs of sections of some flagella.

(*a*) Transverse sections through the tips of flagella of *Pseudotrichonympha* and *Trichonympha*. The series shows the successive reduction in size of the flagellum and the reduction in number of internal fibrils. × 100,000.

(*b*) Longitudinal section through the basal body and proximal part of the flagellum of *Holomastigotoides*. The flagellar shaft is closely applied to the body surface at the left (towards the posterior end of the animal), but not to the right, showing that the insertion of the flagellum is at an acute angle to the body surface. In the shaft the central and peripheral fibrils can be seen, and in the base the three subfibrils of a triplet are visible on the left, while on the right the fibrous ribbon shown in Plate XIIIa is seen in section at the side of another peripheral fibril. × 75,000.

All micrographs are from Gibbons and Grimstone (1960).

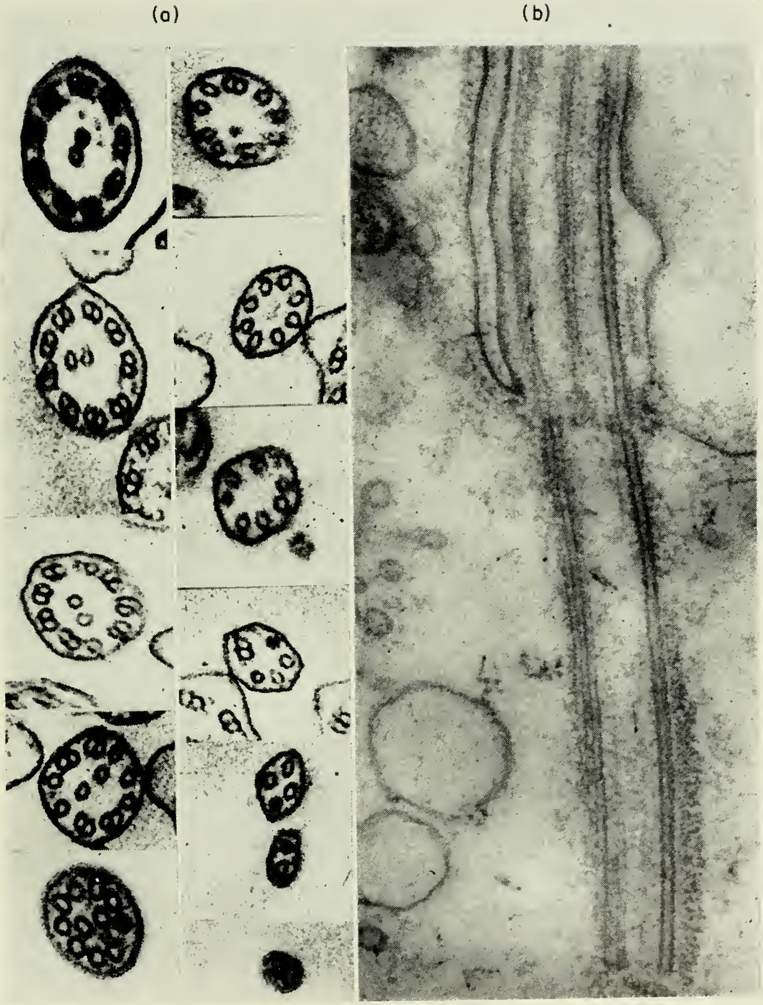
(a)
(b)

PLATE IV. Electron micrographs of sections of basal parts of the membranelles of *Euplotes*.

(*a*) Transverse section showing the double rows of cilia that make up the membranelles. The basal bodies at the lower left are interconnected by fine filaments, and bundles of rootlets run away to the right from the basal bodies. From Roth (1957). × 22,000.

(*b*) Longitudinal section through the basal region of a row of cilia from a membranelle. The central fibrils of the shaft run into a central granule at the level of the cell surface, below which is a transverse plate across the cilium dividing the shaft from the basal body. In the lumen of the basal body is a fibrous structure which may represent continuations of the central fibrils of the shaft. The basal bodies are interconnected at about the middle and at the proximal end, where there are thickenings of the peripheral fibrils. From Roth (1956). × 48,000.

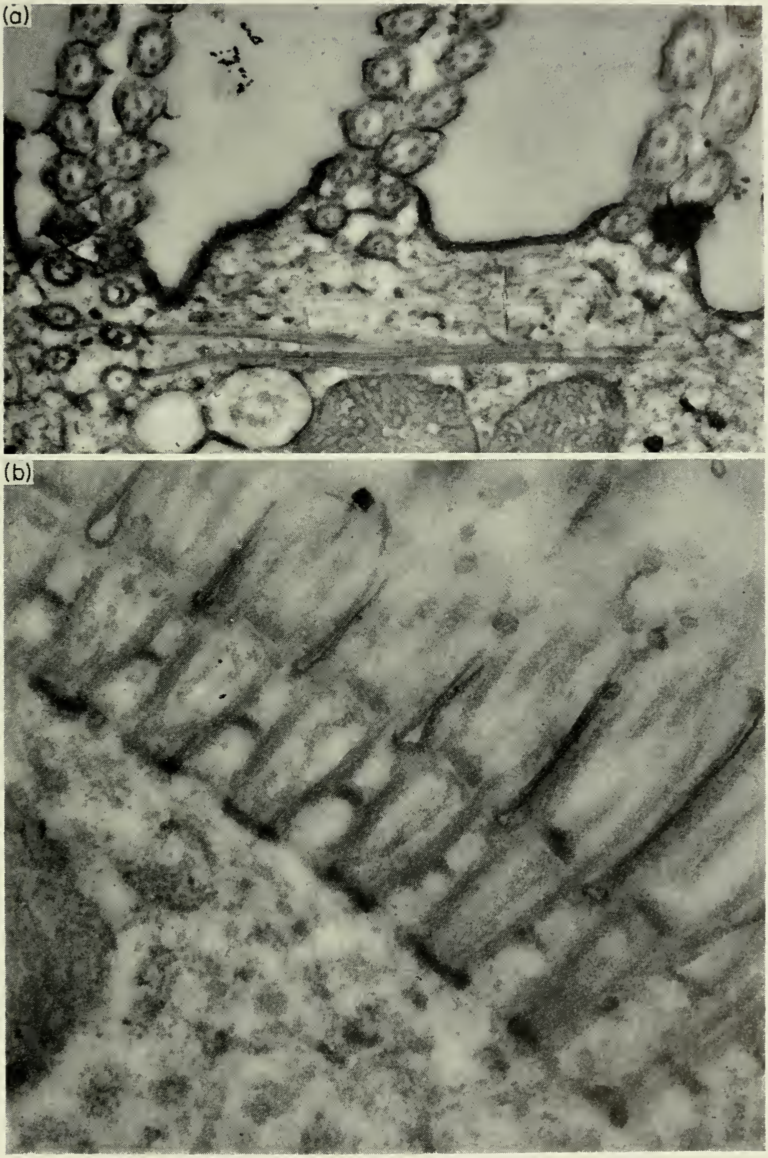
(a)
(b)

PLATE V. Electron micrographs of sections of compound cilia.

(*a*) Transverse section through the base of a cirrus of *Euplotes*, showing the regular arrangement of the cilia and their interconnections. The basal bodies are connected to each other by fine filaments, and those at the edge of the cirrus are joined to bundles of rootlet fibres which diverge to run in various directions just beneath the body surface of the animal. From Roth (1956). × 24,000.

(*b*) Transverse section through several cilia of a comb-plate of *Mnemiopsis*. The cilia show an unusual arrangement centrally, where a " mid-filament " is present between and to the left of the normal central fibrils. Two of the peripheral fibrils (numbers 3 and 8) are connected to the ciliary membrane by " compartmenting lamellae " which terminate exactly opposite similar lamellae of neighbouring cilia, and the lamellae of adjacent cilia may be linked across the gap. The ciliary beat is almost parallel with the longer side of the plate. From Afzelius (1961a). × 155,000.

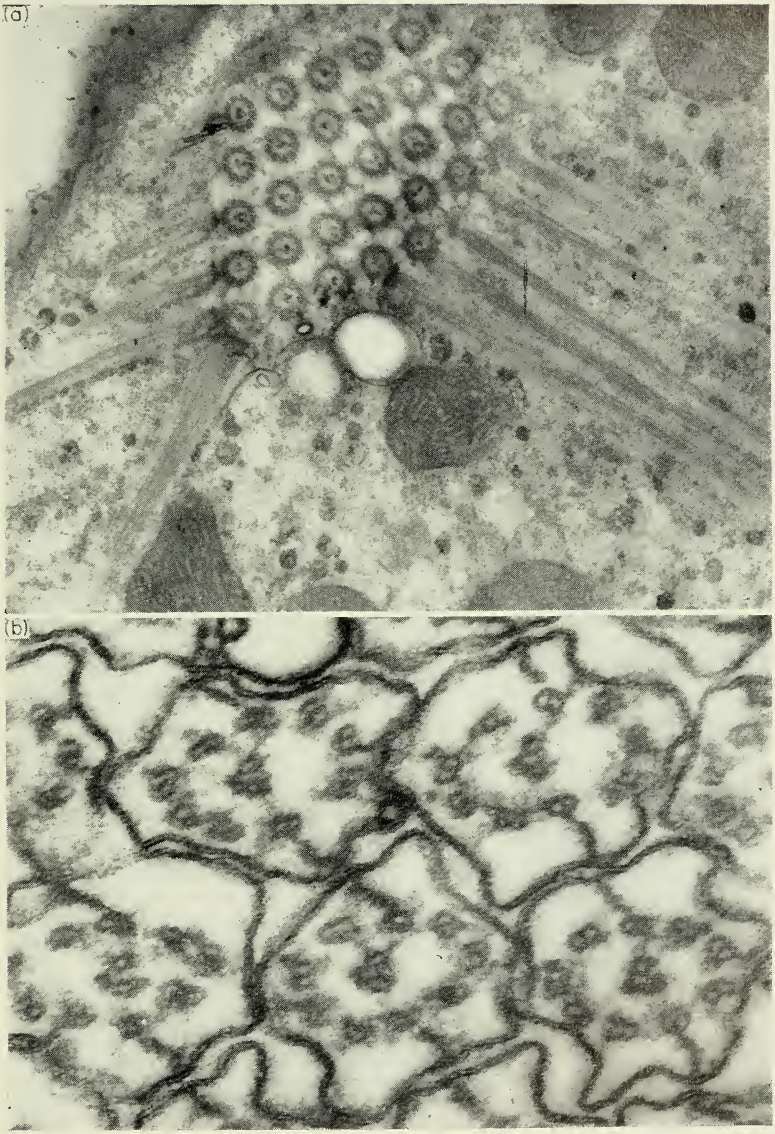
(a)
(b)

PLATE VI. Electron micrographs of sections of sensory cilia.

(*a*) Part of a longitudinal section through a rod cell of the rabbit retina. The inner segment of the cell is connected to the outer segment which contains the flattened sacs by a short ciliary shaft containing fibrils. Below the basal body of the connecting cilium is a second centriole. From De Robertis and Lasansky (1958). × 40,000.

(*b*) Transverse section of the shaft of the connecting cilium of a retinal rod of the rabbit. Note the absence of central fibrils. × 80,000.

(*c*) Transverse section through the basal body of the connecting cilium of the retinal rod of the rabbit. Note the nine groups of fibrils and the club-shaped appendages. × 80,000.

(*b*) and (*c*) from Tokuyasu and Yamada (1959).

(*d*) Longitudinal section through the base of a scolopale cilium of *Locusta* at the apex of the sensory dendrite. The ciliary shaft runs in an extracellular space enclosed by thickened walls of the scolopale structure. Striated ciliary roots run downwards from the basal body of the cilium and pass around a " root apparatus " structure, which may be a second centriole, before joining to form a single large root (see also Fig. 9). From E. G. Gray (1960). × 29,000.

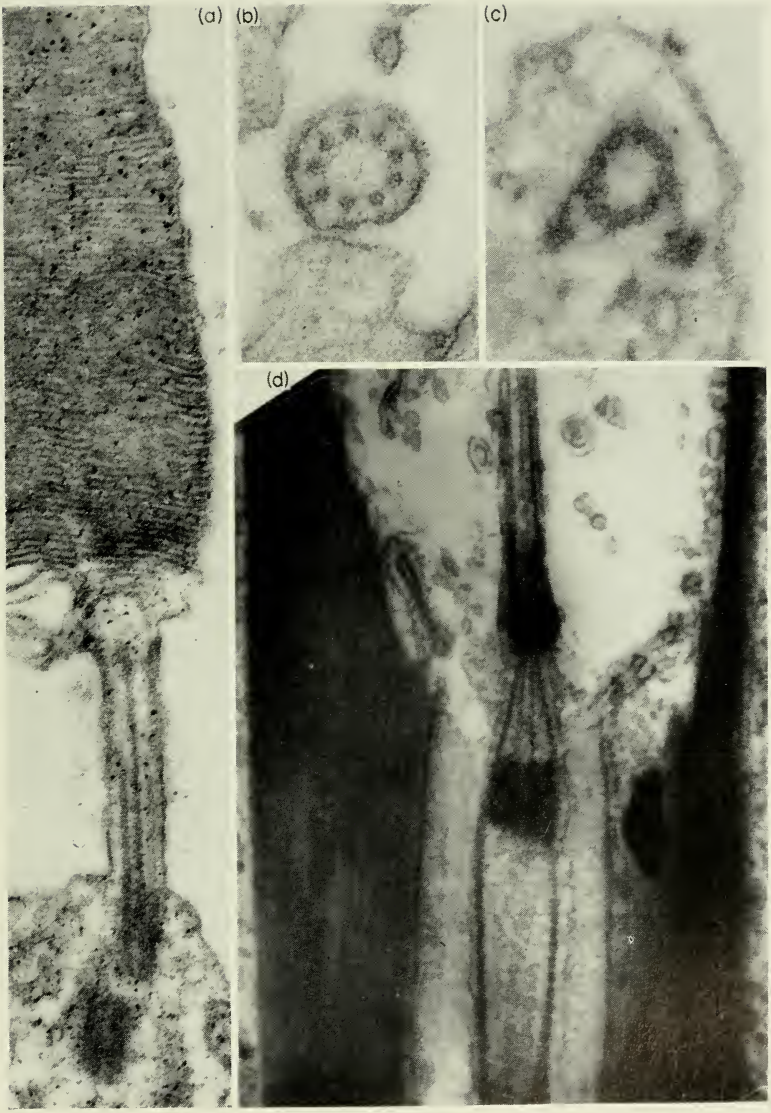
(a)
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PLATE VII. Electron micrographs showing modifications of the shaft structure of flagella and cilia.

(*a*) Transverse section through the flagellar reservoir region of *Peranema*. The two flagellar bases contain normal axial bundles of fibrils and additional strands of intra-flagellar material. × 28,000.

(*b*) Transverse and longitudinal sections of the anterior flagellum of *Peranema*. Within the flagellar membrane is an axial bundle of fibrils and additional intraflagellar material; outside the membrane are two half-cylinders of striated material as well as a zone of fine flimmer filaments. × 30,000.

(*a*) and (*b*) from Roth (1959).

(*c*) Transverse section of the collar region of a choanocyte cell of the sponge *Microciona*. The flagellum carries two rows of fine flimmer filaments in the plane of the central fibrils. The additional structures on the left are sections of some of the filaments that make up the collar. From Afzelius (1961b). × 80,000.

(*d*) Transverse section of the undulating membrane of *Trichomonas termidopsis*. The axial bundle of fibrils is enclosed with some additional material in an expanded flagellar membrane which is attached to a fold of the cell membrane. The dense striated structure within the body is a part of the costa. From Grimstone (1961). × 22,000.

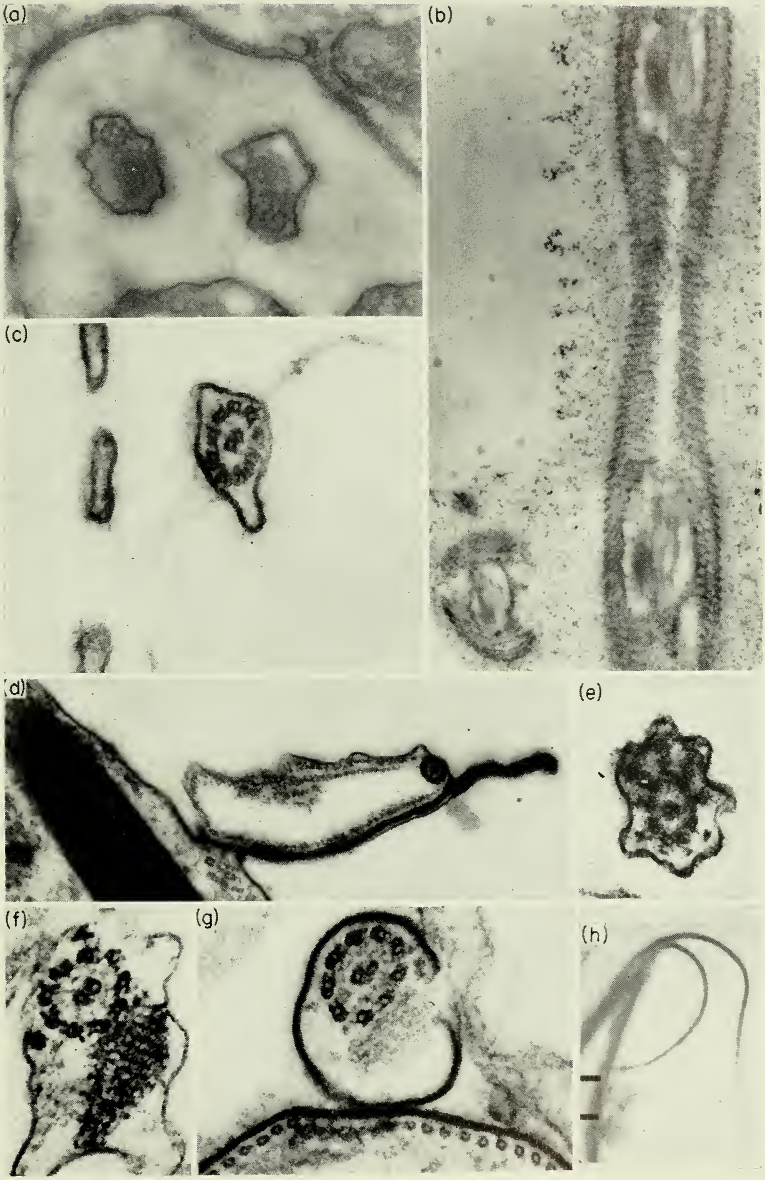
(*e*) Transverse section of a flagellum of *Pyrsonympha vertens*. Additional intraflagellar material is present outside each of the peripheral doublets, and is especially prominent on the side away from the body (at the top). From Grassé (1956). × 50,000.

(*f*) Transverse section of the " undulating membrane " of *Blastocrithidia familiaris*. This part of the flagellar structure is not attached or adherent to the body of the trypanosome. Note that the additional intraflagellar material is striated and in the plane at right angles to the plane through the central fibrils. × 51,000.

(*g*) Transverse section of the " undulating membrane " of *Trypanosoma rhodesiense*. The flagellar structure is here adherent to the body surface, and the additional intraflagellar material is again in the plane at right angles to the plane through the central fibrils of the axial bundle. Note also the fibrils seen in transverse section just beneath the body surface of the trypanosome. × 52,000.

Micrographs (*f*) and (*g*) by Vickerman.

(*h*) The tips of two body cilia of *Stentor polymorphus*. The basal region of the shaft has a normal diameter, as seen on the left, but the terminal part is narrowed to form a " whiplash." Original. × 8000.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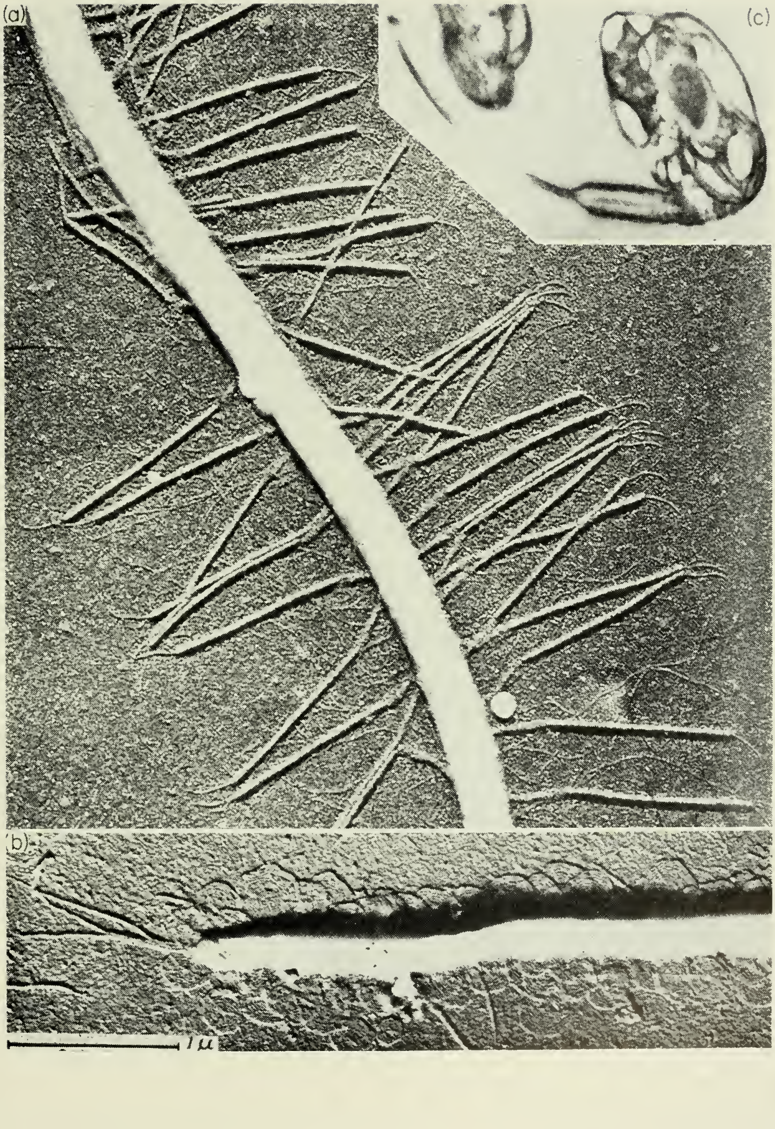
(h)

PLATE VIII. Electron micrographs of flagella of some phytoflagellates.

(*a*) Part of a flagellum of *Ochromonas* showing the mastigonemes which carry one or more fine filaments at their ends. Some of the fine flimmer filaments are also visible. From Pitelka and Schooley (1955). × 27,000.

(*b*) The tip of a flagellum of *Micromonas squamata* showing hairs and scales attached to and detached from the flagellum. From Manton and Parke (1960). × 22,000.

(*c*) Section through the whole body of *Chromulina pusilla*, including a longitudinal section through the flagellum. The short basal part of the flagellum contains the full 9-plus-2 bundle of fibrils, while the thin whiplash tip contains only the two central fibrils. From Manton (1959). × 26,000.


(a)
(c)
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1 μ

PLATE IX. Electron micrographs of flagella and haptonemata from algae.

(*a*) The cell body and the bases of the two flagella of a spermatozoid of *Fucus serratus*. The anterior flagellum (on the right) carries two rows of mastigonemes, while the posterior flagellum is bare. The two flagella diverge from their bases near the anterior end of the cell, and the posterior flagellum returns to the cell surface near the middle of the body where it adheres to the surface of the eyespot. From the region of the flagellar bases the fibrils that make up the " proboscis " run out to form a flat loop at the anterior end. From Manton and Clarke (1951). × 15,000.

(*b*) The distal end of the anterior flagellum of *Himanthalia*. The two rows of mastigonemes which occur on the main part of the flagellar shaft are absent beyond the large spine. × 7,500.

(*c*) The tip of a dismembered anterior flagellum of *Himanthalia*. Eleven fibrils can be distinguished, one of which carries a large spine. Mastigonemes can also be seen, but their relationship to the fibrils is uncertain. × 5,500.

(*b*) and (*c*) are from Manton, Clarke and Greenwood (1953).

(*d*) A whole cell of *Chrysochromulina strobilus* showing the two flagella and the long coiled haptonema. × 1,800.

(*e*) Transverse section of a haptonema of *C. strobilus* showing the six internal fibrils surrounded by three concentric membranes. × 92,000.

(*d*) and (*e*) are from Parke, Manton and Clarke (1959).

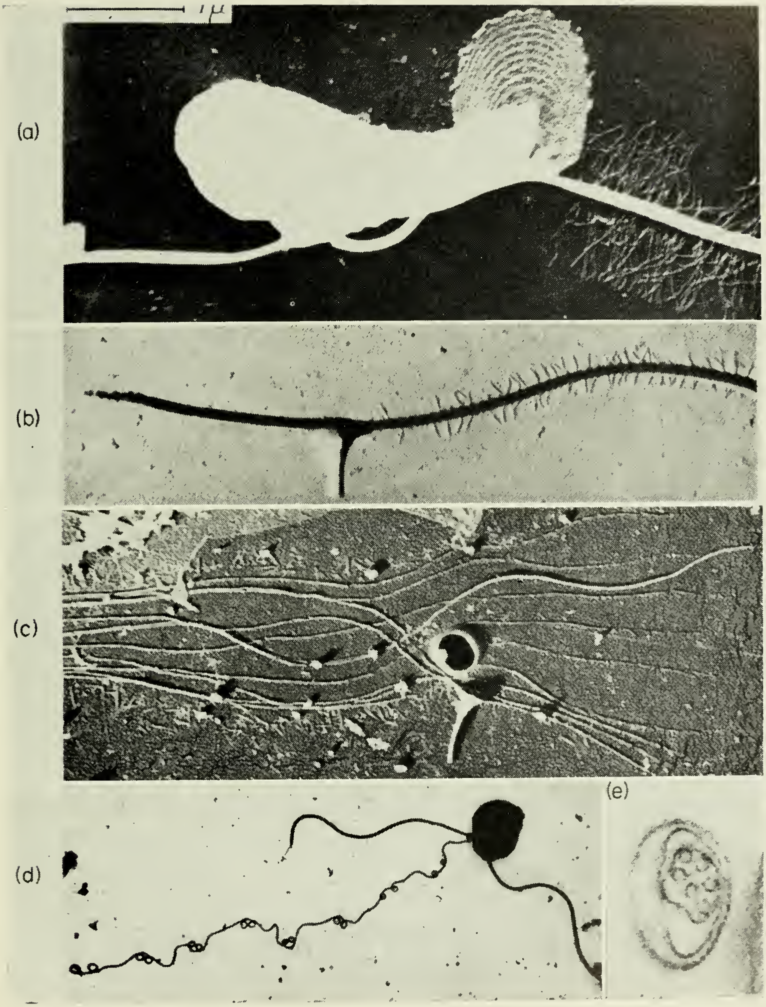
1 μ
(a)
(b)
(c)
(d)
(e)

PLATE X. Electron micrographs of some sections of sperm tails of invertebrates.

(*a*) Transverse section through the tail of a sperm of *Psammechinus*. Arms can be seen on one subfibril of each doublet, and a more complex bridge is seen at the lower left (between fibrils 5 and 6). Radial spokes can be seen connecting the central fibrils to some of the doublets; note that these spokes run to the subfibril that bears the arms, and that this subfibril appears slightly larger than the other, armless, subfibril. From Afzelius (1959). × 220,000.

(*b*) Parts of a longitudinal section through the axial fibre bundle of a sperm tail of *Haematoloechus*. At the right the central structure of the bundle can be seen to be connected to the peripheral fibrils lying on either side by a series of spokes. In places a faint dense spiral in the central structure may be associated with the spokes. × 160,000.

(*c*) Parts of several transverse sections of sperm tails of *Haematoloechus*. At the right is an abnormal tail which carries two axial fibril bundles surrounded by an incomplete ring of longitudinal fibrils just inside the cell membrane. The central structure of the axial bundle is seen to be composed of a central dense region surrounded by first a less dense zone and then another dense zone. Prominent radial spokes connect this outer dense zone to the armed subfibrils of the peripheral doublets. The armed subfibrils appear to be solid. × 200,000.

(*b*) and (*c*) are from Shapiro, Hershenov and Tulloch (1961).

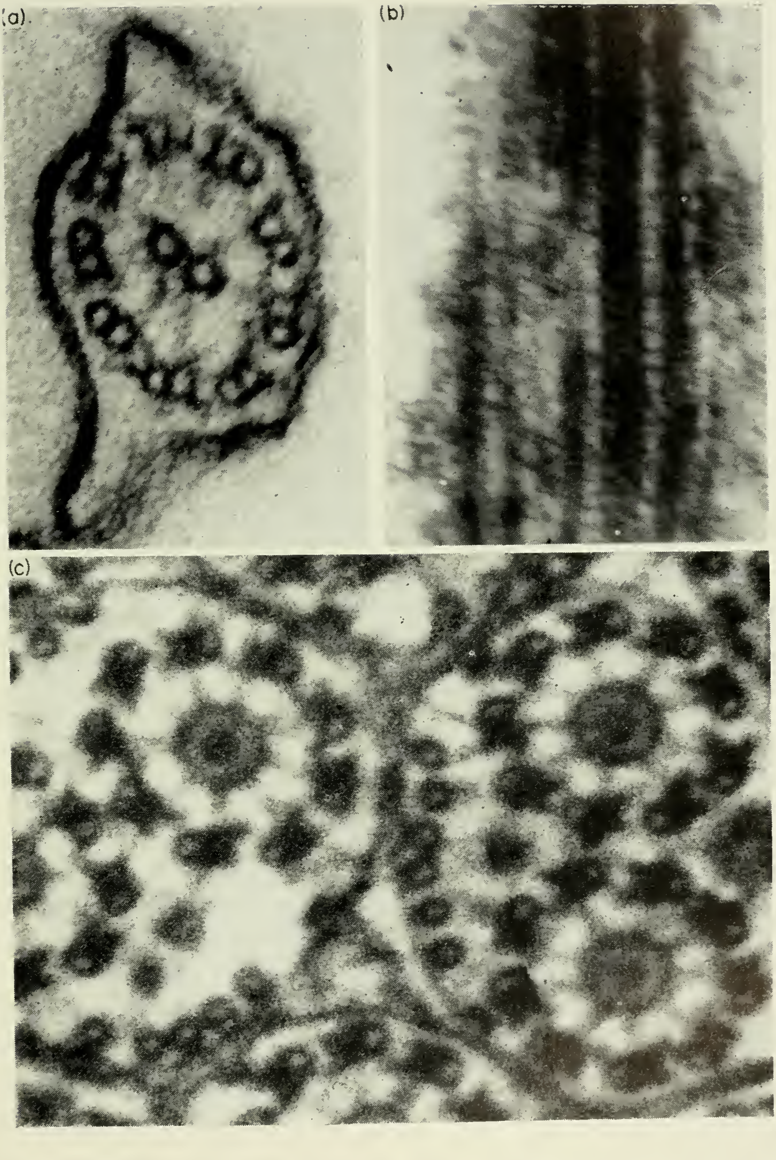
(a)
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PLATE XI. Electron micrographs of transverse sections of sperm tails.

(*a*) Sections of two sperm tails of *Bufo*. The main axis of the tail contains the 9-plus-2 bundle of fibrils. A fin (undulating membrane) of dense material extends to one side in the plane of the central fibrils and ends at a thickened margin. From Burgos and Fawcett (1956). × 60,000.

(*b*) Incomplete section of a sperm tail of *Apis*. The surface membrane of the cell is missing, but the remaining structure appears to be complete. The two large structures on the right are the longitudinal mitochondrial strands; they are separated from the axial fibre bundle by two additional structures of unknown function. The peripheral fibrils of the axial bundle have outward extensions of dense material, and surround some interesting structures including the central fibrils which are linked by a semicircular filament. From Rothschild (1955). × 110,000.

(*c*) Transverse sections through the principal-piece region of three sperm tails of *Cavia*. The 9-plus-2 fibril bundle is clearly seen, and shows many details of fine structure, such as radial links and connexions between the central fibrils. One subfibril of each peripheral doublet appears to have a dense centre, and also bears the arms. Seven outer coarse fibres are prominent in each section, and each bundle is surrounded by a dense fibrous sheath, from which laminae project towards peripheral fibrils 3 and 8. The cell membrane is separated from the fibrous sheath by a less dense region. Micrograph by Fawcett. × 100,000.

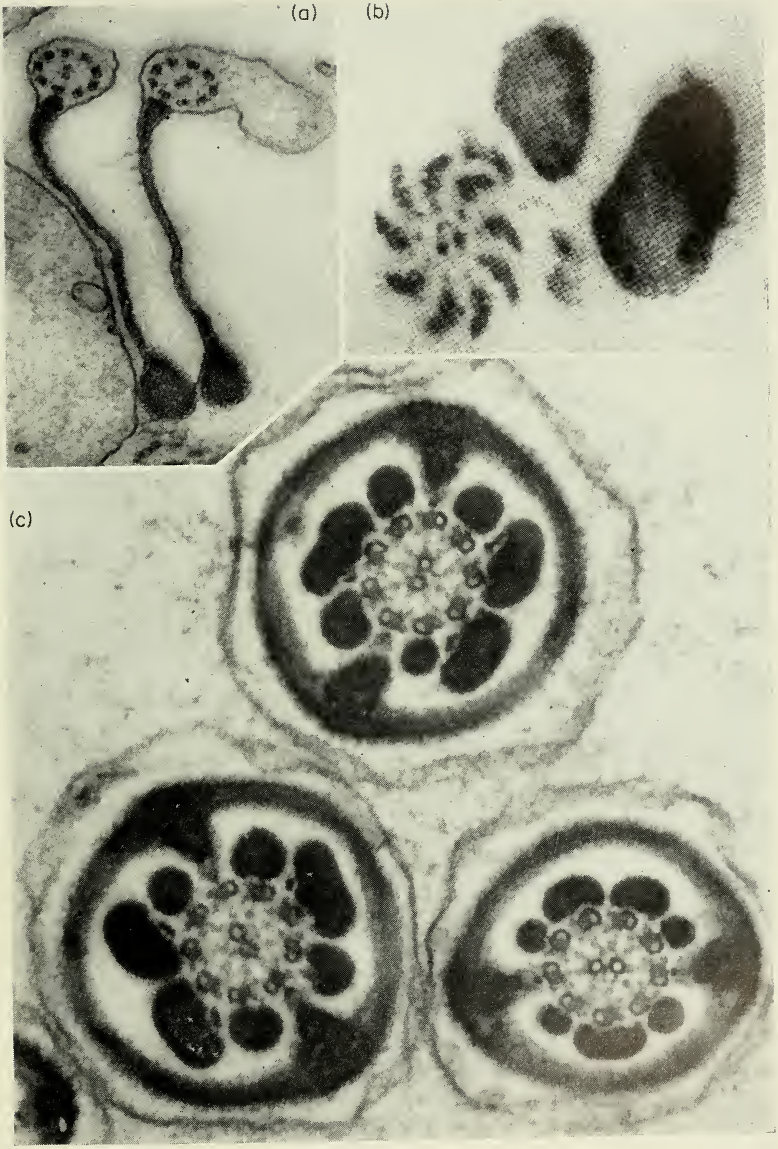
(a)
(b)
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Plate XII. Electron micrographs of some centrioles and basal bodies.

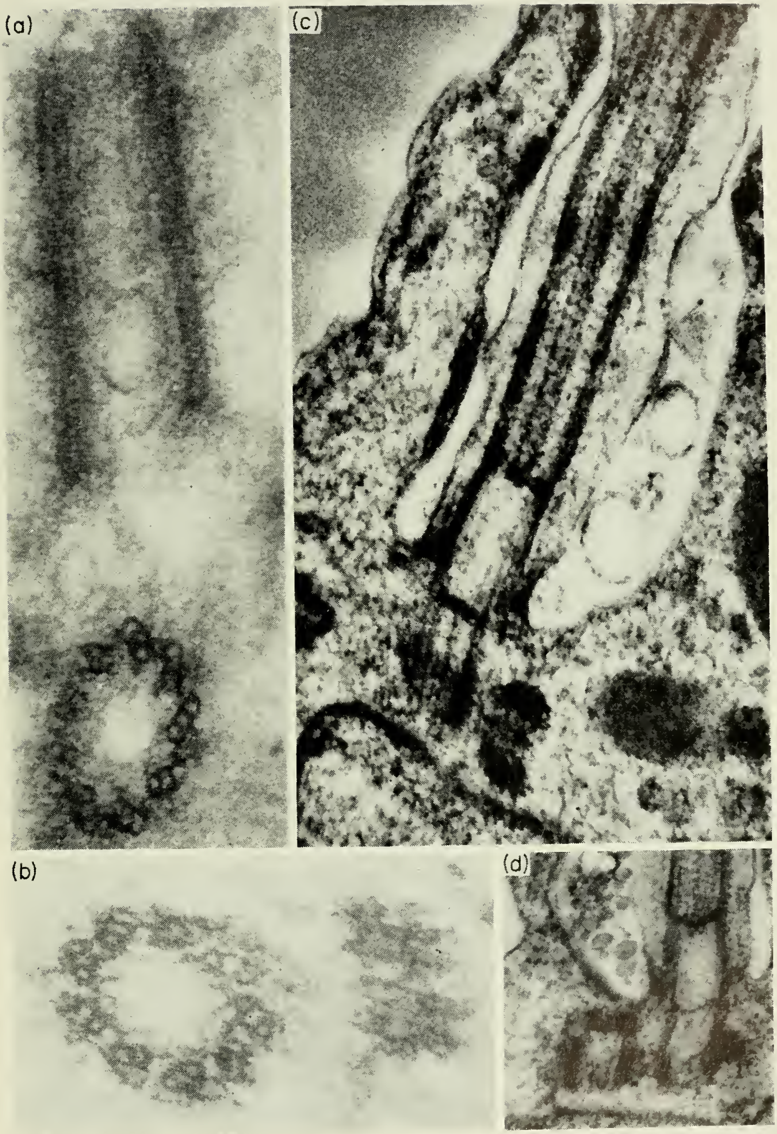
(*a*) A pair of centrioles from a human lymphosarcoma cell. The centrioles are cut in longitudinal (above) and transverse section, and lie in a characteristic position with their axes at right angles. Note the triplets of fibrils seen in the transverse section, and compare their arrangement and interconnexions with those seen in Plates I and XIIIa. Micrograph by Bernhard. × 145,000.

(*b*) A stage in the duplication of a centriole of a *Viviparus* spermatocyte. The parent centriole is shown in transverse section, and the short procentriole is seen in longitudinal section growing at right angles to its parent. From Gall (1961). × 145,000.

(*c*) Longitudinal section through the basal part of a flagellum of *Blastocrithidia familiaris*. The basal region of the flagellar shaft lies in a reservoir at the anterior end of the flagellate. It appears from some transverse sections that there may be a complex transition zone between the shaft and the basal body; this probably extends from near the proximal end of the central fibrils through the part with the less dense centre to the basal body proper, which contains fibril triplets and a cartwheel structure of hub and spokes. To the right of the basal body is seen a short daughter basal body orientated at right angles to its parent. × 56,000.

(*d*) Longitudinal section of a basal body of *B. familiaris* with a developing daughter basal body. The structure of the transition zone of the flagellar base is again seen. The daughter basal body has increased in length, compared with that in (*c*), and has turned to lie parallel to its parent. × 38,000.

Micrographs (*c*) and (*d*) by Vickerman.


(a)
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(d)

PLATE XIII. Electron micrographs of basal bodies and root connexions of some protozoa.

(*a*) Transverse section through a row of basal bodies of *Holomastigotoides*. The flagellar bases of the spiral row are connected together by a fibrous band which runs on the (animal's) right-hand side of the row. Note also the arrangement of the fibril triplets and their interconnexions. From Gibbons and Grimstone (1960). × 70,000.

(*b*) Transverse section through several basal bodies of a kinety of *Stentor*. The root fibrils that are attached to the basal bodies join many similar fibrils to form a large longitudinal bundle. The top of the plate is anterior, and the fibrils run posteriorly from the basal bodies. From Randall and Jackson (1958). × 24,000.

(*c*) Transverse section through the basal bodies of a row of cilia of *Opalina*. Short curved fibrils connect the basal bodies in an asymmetrical way and confer some polarity on the row. From Pitelka (1956). × 23,000.

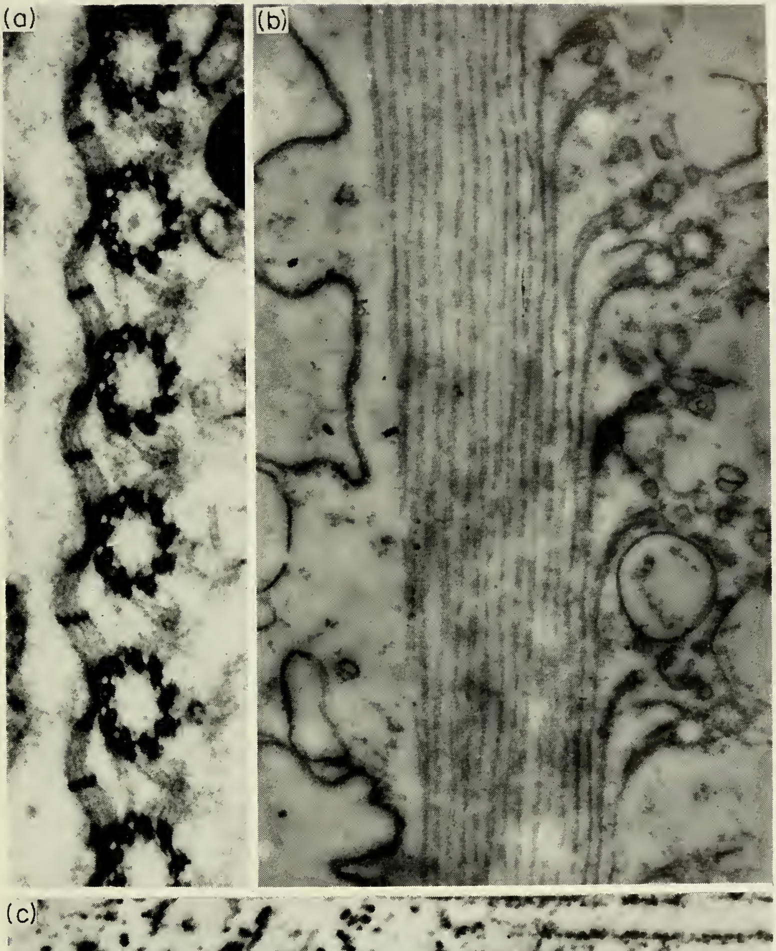
(a)
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Plate XIV. Electron micrographs of striated roots of metazoan cilia.

(*a*) Longitudinal section of part of a ciliated cell from the typhlosole of *Elliptio*. A striated root bifurcates just below the basal body and runs deep into the cell. From Fawcett (1958). × 60,000.

(*b*) Longitudinal section of a small part of the root of a locust scolopale cilium. The striation pattern can be subdivided into about six intraperiod bands. From Gray (1960). × 50,000.

(*c*) Longitudinal section of a short length of a ciliary rootlet of *Anodonta*. About twelve intraperiod striations appear to be present in this case, and the striation pattern is polarized. Some of the fine longitudinal filaments that make up the rootlet are faintly visible. From Gibbons (1961b). × 175,000.

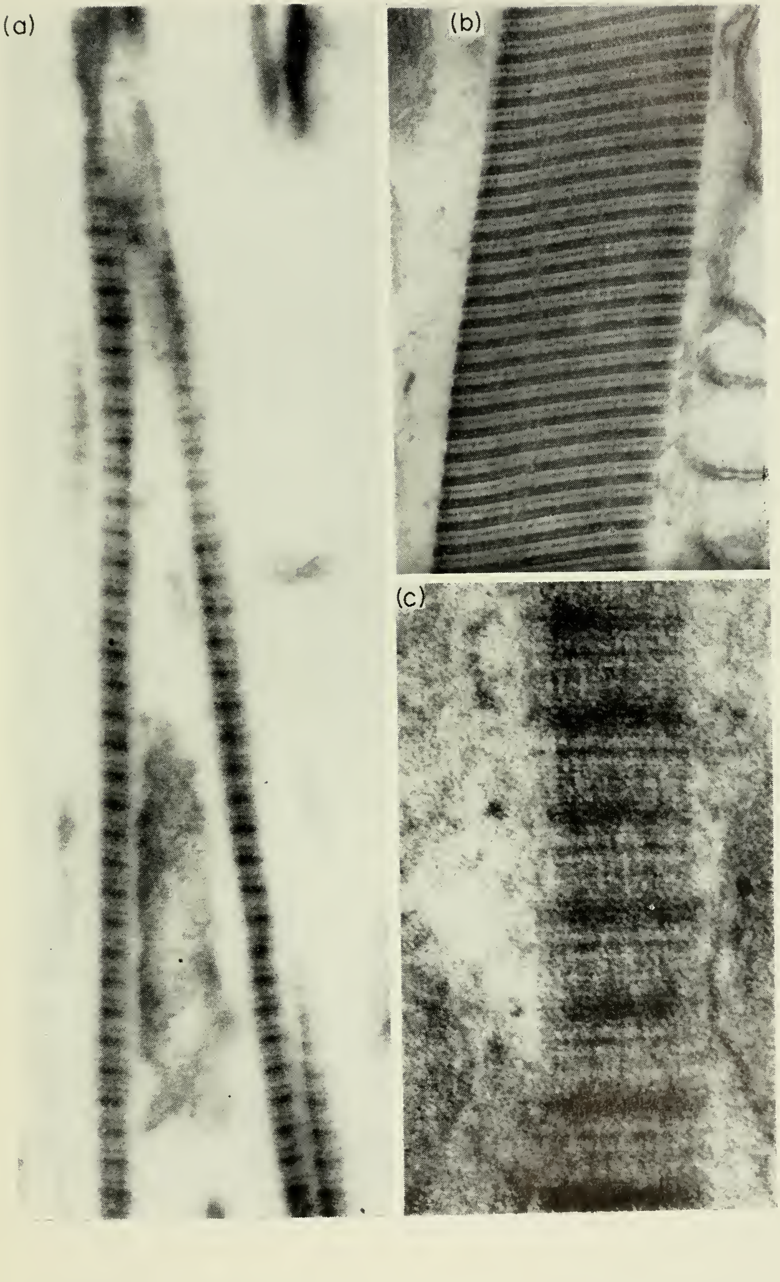
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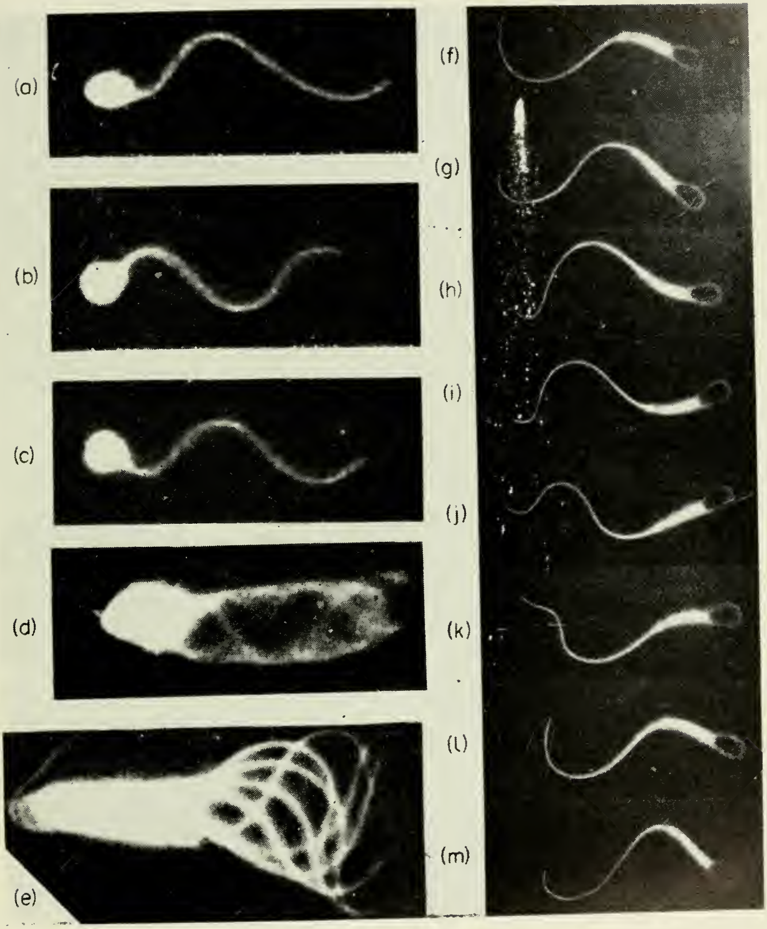
Plate XV. The movement of spermatozoa of the sea urchin and bull.

(*a*, *b* and *c*) Photographs of sea urchin sperm showing the form of the bending waves of the tail. (Exposure $\frac{1}{500}$ sec). From Gray (1955). About × 1000.

(*d*) Photograph of a sea urchin sperm showing the " optical envelope " traced out by the moving tail. (Exposure $\frac{1}{25}$ sec). From Gray (1955). About × 1000.

(*e*) A succession of photographs of a moving bull sperm at $\frac{1}{80}$ sec intervals, showing the " optical envelope " traced out by the tail. From Gray (1958). About × 800.

(*f–m*) Eight frames of a cine film of a moving bull sperm taken at intervals of $\frac{1}{66}$ sec. From Gray (1958). About × 500.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(g)
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(i)
(j)
(k)
(l)
(m)

PLATE XVI. Photographs to illustrate the movement of two polyflagellate protozoa.

(*a*) A series of photographs (taken from a cine film) showing the movement of the anterior flagella of *Trichomonas termidopsis*. All of the anterior flagella were moving together in this case. The prints have been retouched to emphasize the shape of the flagella, which are otherwise only faintly visible. Note that the beat is not unlike that of a cilium—it is unidirectional and shows only one bending wave within its length.

The undulating membrane runs most of the way around the edge of the animal in these prints, having its origin near the base of the anterior flagella and ending near the projecting tip of the axostyle, which here lies close to the flagellar bases. In most of the prints the waves of movement of the undulating membrane can be seen; the movement of one wave is indicated by the arrows.

The interval between the frames is $\frac{1}{12}$ sec. × 170.

(*b*) Photograph of a living *Trichonympha*. The flagella move in waves which pass backwards down the body from the rostrum at the anterior end. Four of these waves are indicated by arrows. × 250.

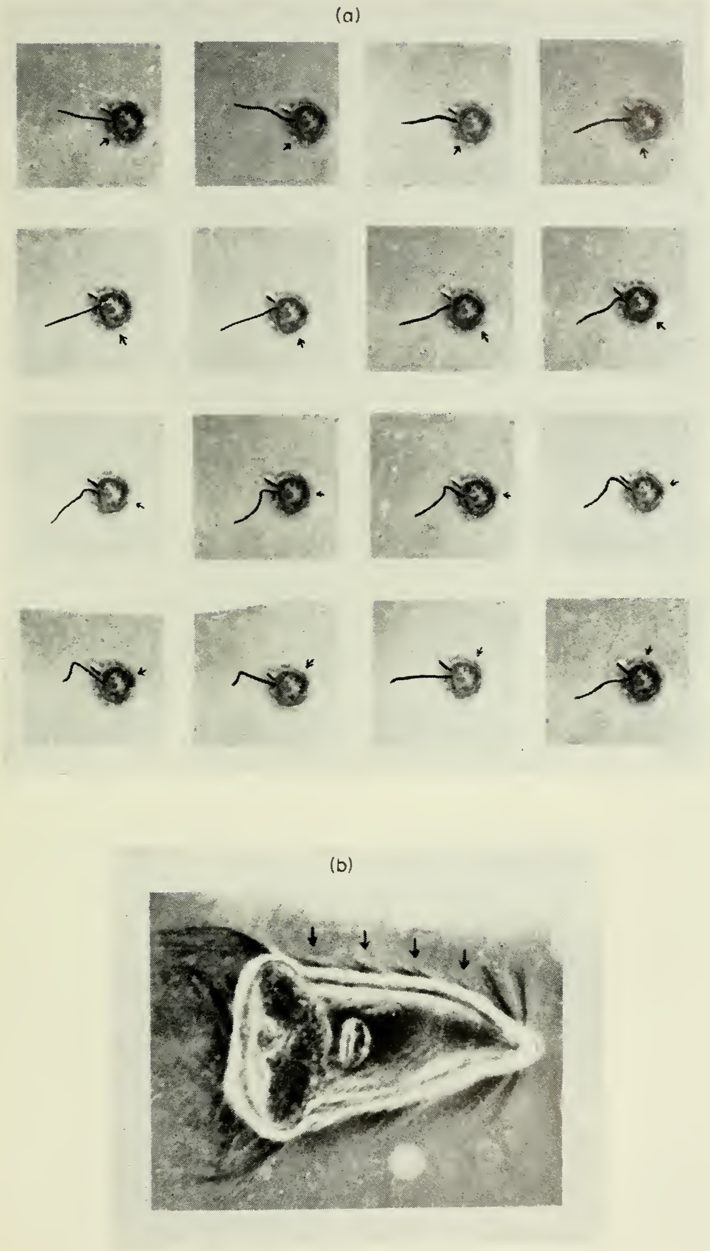
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PLATE XVII. The movement of two types of cilia from *Mytilus* gill filaments.

(*a*) Two cycles of beating of an abfrontal cilium. Note the quick " flick " from the resting position at the start of the movement to the upright position, and the slow return to the gill surface. × 100.

(*b*) One cycle of beat of two large frontal cilia. The two cilia lie close together and beat more or less in unison. The recovery phase of the beat takes considerably longer than the effective phase. × 100.

Both sequences filmed at 24 frames per sec; prints have been retouched to enhance contrast.

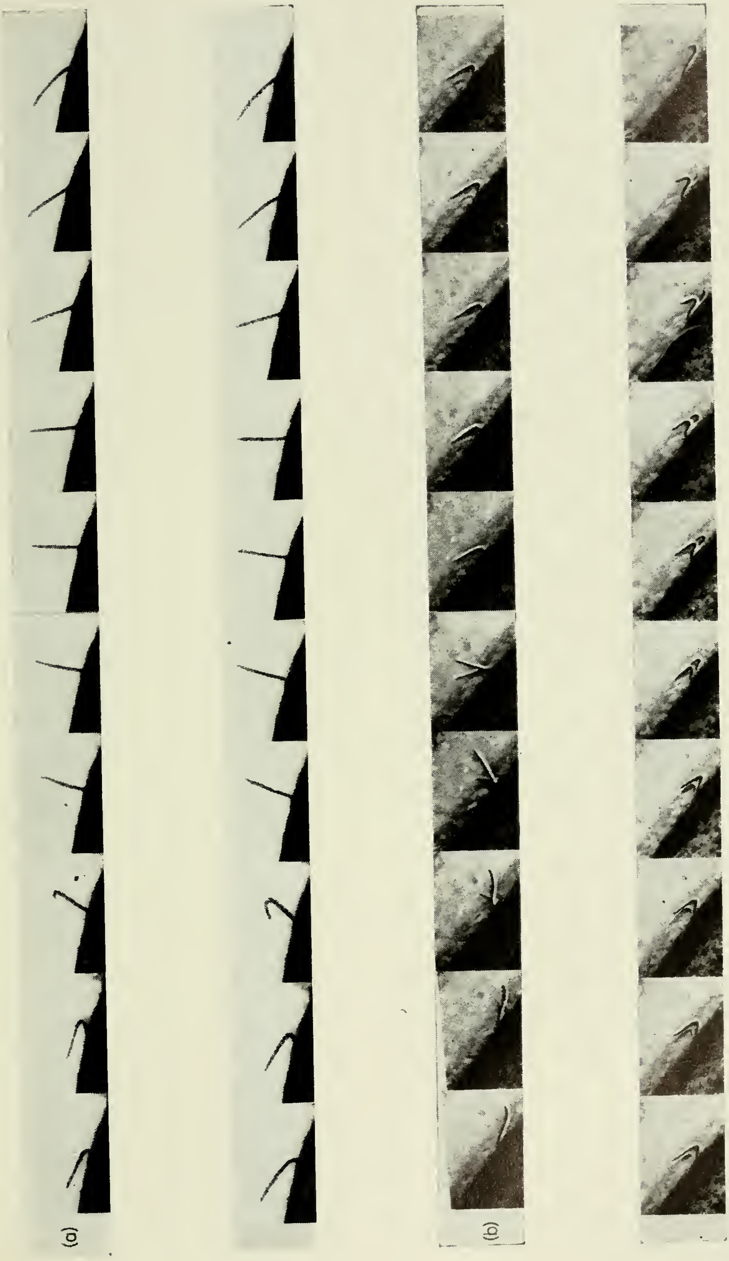
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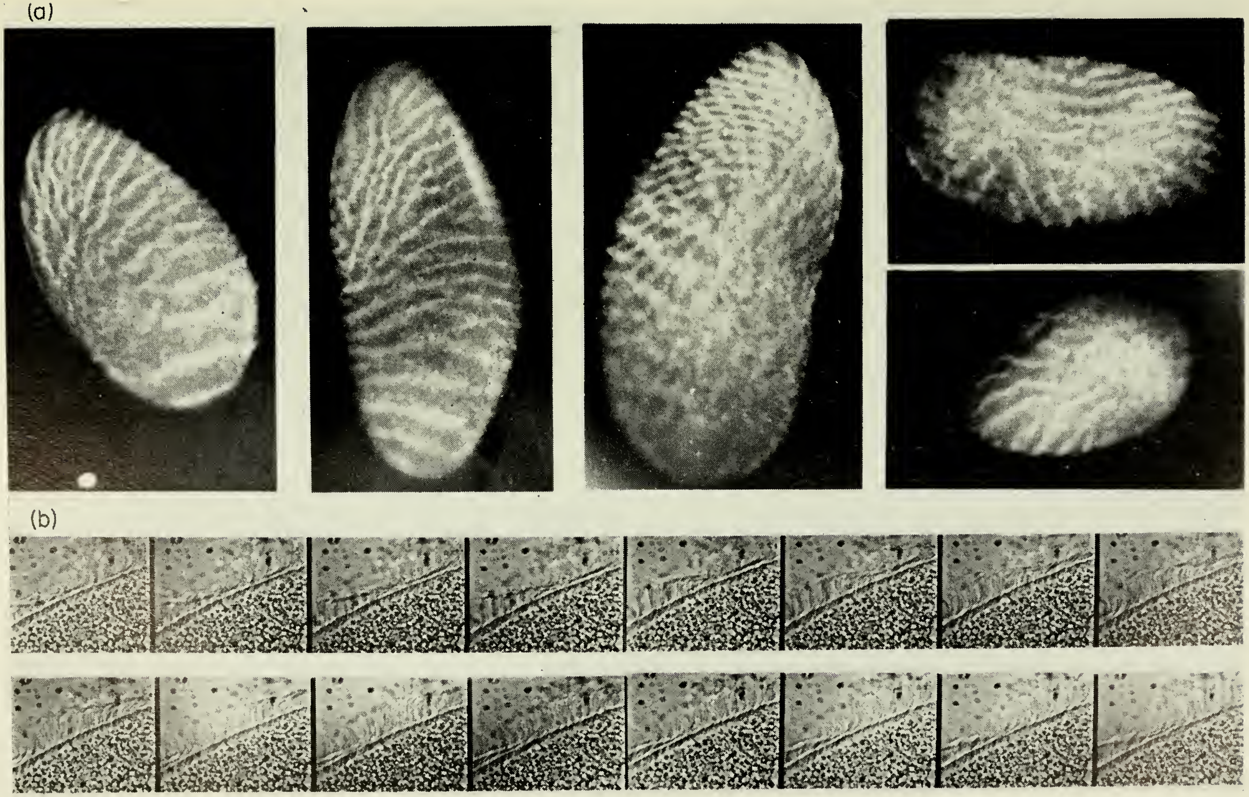
PLATE XVIII. Metachronal waves and ciliary beating of *Opalina*.

(*a*) Dark-ground photographs of several animals to show examples of metachronal wave patterns. The two animals on the right are turning; in all cases the waves are moving approximately from the top of the plate to the bottom. All prints at about × 40.

(*b*) A series of cine photographs of a group of cilia of *Opalina*. The metachronal waves move from left to right along the row of cilia that are in focus. (Cf. Figs. 39 and 47.) Interval between exposures $\frac{1}{24}$ sec. × 300.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